ANNUAL PROGRESS REPORT

NASA Grant NsG 441-63

Title of Project: Integrated Research and Training in Space-Molecular Biology

Period of Project: April 1, 1964 - March 31, 1965

Institution: The University of Chicago

Principal Investigator: Humberto Fernández-Morán, M.D., Ph.D.
Professor of Biophysics
Department of Biophysics

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Principal Investigator	Grant Number
Dr. Humberto Fernández-Morán	NsG 441-63
Institution	**Period of Project**
The University of Chicago	April 1, 1964 through March 31, 1965

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Integrated Research and Training in Space-Molecular Biology

Following the program set forth in our research proposal, and as described in the enclosed reports and reprints, our efforts during the past year have centered on:

I. Completion of Organization, Testing, and Operation of the Electron Microscope Laboratories and Adjacent Laboratories (Rooms 203B, 205, 207) for the Proposed Research and Training Program.

A. With funds provided by grant NsG 441-63 from the National Aeronautics and Space Administration, by grant USPHS MED RES 943 from the National Institutes of Health, by grant AT (11-1) 1344 from the Atomic Energy Commission, and by funds from The University of Chicago, including Otho Sprague Institute and L. Block funds, the following alterations and additions were made in the Adjacent Laboratories (Rooms 203B, 205, 207):

1. Room 203B (230 sq. ft.)

a. Preparation of room for storage of specimens, equipment, and laboratory apparatus.
b. Installation of Harris Cascade Refrigeration Biological Storage Machine which operates at temperatures as low as -120°C.

2. Room 205 (230 sq. ft.)

a. Installation of Siemens Elmiskop EM-II with accessories.
b. Installation of darkroom equipment to expedite development of plates taken during experiments in this room.
c. Preparation of room as site of superconducting experiments.

3. Room 207 (460 sq. ft.)

   a. Installation of X-Ray diffraction unit with Kratky Camera.
   b. Development and installation of 2 vacuum pumping units used to pre-pump photographic plates (at a rate of 912 plates per 2 hours), also with capacity to pre-pump 70mm film and camera. The efficiency of these pumps is such that it reduces working time by several hours. Plates, film, and camera were previsouly pre-pumped in the microscope itself which involves a much longer time.

4. Clean Room Laboratory E-III

   a. Construction of special laboratory in Room P-III, adjacent to darkroom to house Hitachi Perkin-Elmer microscope and accessories.
   b. Installation of High Performance Hitachi Perkin-Elmer Electron Microscope and accessories, including Double Condenser Lens, Electron Diffraction Chamber, Hot and Cold Stages and Image Intensification System.

B. With the same funds mentioned above, the following equipment was added to our facilities:

1. Power supply for superconducting solenoids
2. Air Core dewar and coil support
3. Superconducting solenoids
4. Photographic Equipment: Enlarger and Accessories
   Mercury Arc Light Source
   (Durst, Inc.)
5. Rotary Vacuum Pump and Accessories (Varian)
6. Microscope Vacuum Heating Stage (Leitz)
7. Diffusion Pumps
8. Precision Voltmeter-Ohmmeter and Ammeter and Oscilloscope
9. Control Unit (Varian)
10. Electron Diffraction and X-Ray Diffraction Units

All of the laboratories are now fully operational. Extensive tests demonstrated the exceptional level of high resolution, of the order of 4 to 6Å point resolution, which can be consistently achieved. We have been able, for the first time, to attain resolutions of 4 to 6.9Å in certain crystals (K_2PtCl_4), recorded at low temperatures (-170°C) with the special cold stage which was developed at our suggestion for the Hitachi 11-B electron microscope.

Dr. Keiji Yada, a visiting research associate from Japan, has also, for the first time, been able to achieve 2.8Å resolution, imaging the (200) plane of NaCl crystals by direct illumination using a special pointed filament with tantalum tip of the Moran type. This represents an important improvement over the previously recorded value of about 4Å.

These laboratories and the additional facilities are generally considered to be the most advanced facilities available for high-resolution electron microscopy. Many of the features developed for the first time here in our laboratories are being incorporated in other major laboratories in the United States and in Europe.

II. Specific Research Program.

A. Continuation of correlated electron microscope and electron diffraction studies of certain meteorites (Orgueil carbonaceous chondrite) carried out with Dr. Edward Anders and Dr. Frank W. Fitch of The University of Chicago. Preliminary experiments indicate that the composition and structural relationships of its constituents can be determined by the high resolving power of the electron microscope. Various preparation techniques are being applied under carefully controlled conditions. These techniques include ultrathin sectioning with a diamond knife, mechanical and selective chemical dissociation followed by density gradient separation, negative staining, shadow-casting, etc. The ultrastructural data will be correlated with parallel chemical studies of organic constituents and with the results of selected area electron diffraction analysis.

B. Continuation of electron microscopical studies of PreCambrian organized systems. Preliminary investigations of nonferruginous cherts of the Gunflint formation of southern Ontario were carried out by electron microscopy in collaboration with Dr. Edward Anders and Dr. F. Fitch. Dr. S. A. Tyler of the University of Wisconsin and Dr. E. Barghoorn of Harvard University have reported (Science, Vol. 119, p. 606, 1954) the occurrence of

primitive lower plants in these PreCambrian rocks, which are the oldest (about 2 billion years) structurally preserved organisms that clearly exhibit cellular differentiation. Electron microscopy reveals the presence of filaments, tubular structures and membranes of apparent organic origin.

A comprehensive report on this work is being prepared. Electron micrographs taken during these studies were reproduced in the 1965 McGraw-Hill Yearbook of Science and Technology under section entitled Photographic Highlights, p. 86.

These studies are of great interest in the evolutionary scheme of primitive life, since they may furnish insight into the molecular organization of the oldest known preserved living systems, bearing also on the evolution of membrane ultrastructure.

C. A comprehensive experimental program of electron microscopy using high-field superconducting solenoid lenses was initiated.

The resolving power of the electron microscope has extended the range of direct visualization to structural details of the order of a few angstroms. This corresponds to the size of small molecules and to the atomic spacing in crystalline lattices. However, although the wavelength of electrons in standard microscopes is 100,000 times shorter than the wavelength of light, the best electromagnetic and electrostatic lenses available have usable apertures limited, by aberrations, to semiangles of the order of 1/100 radian as compared with the numerical apertures of 1.5 of the best light microscope lenses. Considering the numerous complex instrumental and preparative factors involved, the major steps which have to be taken for attainment of the ultimate theoretical resolution are correction of lens aberrations (mainly spherical and chromatic aberrations), stabilization of the lens excitation current, and accelerating voltage. Thus, the degree of stability required for very high resolving powers (in the range of 4Å) is of the order of 1 to 2 parts per million, since the focal length of a magnetic lens is dependent on the electron energy as well as on the lens excitation current. In addition, if the present limitations of the strength and configuration of the axially symmetrical field formed by iron pole pieces could be overcome, "stronger lenses" of shorter focal length could be designed with correspondingly reduced aberrations.

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With the introduction and availability of new high-field superconducting solenoids of alloys of niobium-zirconium and niobium-tin, it is now possible to obtain magnetic fields in excess of 60 kilogauss over relatively large volumes when operating at liquid helium temperatures, without the continual expenditure of vast amounts of electrical power. It has been demonstrated that operation of superconducting solenoid, short-circuited, or in "the persistent current mode," yields large uniform magnetic fields which are highly homogeneous to better than one part in 10^6 to 10^7, and are highly stable and noise-free under appropriately controlled conditions.

1. Based on previous work in low-temperature electron microscopy, preliminary experiments have been successfully carried out with a simple electron microscope which can be used for transmission electron microscopy and electron diffraction, using high-field superconducting solenoid lenses in an open-air-core, liquid helium Dewar, preferably operating in the persistent current mode. In a series of controlled, reproducible experiments, electron microscopic images have been recorded of test specimens with accelerating potentials of 4 to 8 kV, using a niobium-zirconium solenoid without pole pieces, operating at 32.2 kilogauss in the persistent current mode. These preliminary experiments demonstrated, over a period of 4 to 8 hours of continuous operation, the exceptional stability of the images and also their relatively high quality at magnifications of 50 to 100 X. Details and results of this work, which is generally regarded as the first of its kind, are reported in the following publication: Fernandez-Moran, H., Electron Microscopy with High-Field Superconducting Solenoid Lenses, Proc. of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451. February, 1965.

2. Further experiments have been carried out with various types of electron microscopes using high-field superconducting solenoid lenses and accelerating voltages of 50,000 volts. These experiments have demonstrated the exceptional stability of the images and their relatively high quality under carefully controlled conditions. The results obtained with these experiments and the observations on imaging phenomena with superconducting solenoid lenses are providing

essential data for the design of new types of miniaturized electron microscopes immersed in a liquid helium cryostat. Potential advantages of this promising approach to high resolution electron microscopy at cryogenic temperatures, as well as present methodological limitations were discussed during the Annual Meeting of the National Academy of Sciences. The described combination of optimized instrumental design parameters operative under conditions of minimized specimen perturbation represents one of the most promising coherent experimental approaches towards attainment of the theoretical resolution limit (about 2Å) in direct examination of organic and biological structures. This work was reported at the Annual Meeting of the National Academy of Sciences, April 26-28, Washington, D.C. and published in Science, Vol. 148: 665. April, 1965.

D. The principal investigator participated with the National Academy of Sciences in the Exobiology Study on the Biological Exploration of Mars, Stanford University, California, 1964. Following these meetings, the investigator wrote two papers in connection with the Study.

1. In the first paper, entitled "Analytical Systems for Biological Study of Mars: The role of electron microscopy and electron optical techniques in Exobiology" it is proposed that the electron microscope, and the microscope in its broadest sense, may prove to be the prime analytical tool, both for the detection of "entropy pockets" in an alien planetary environment, and for subsequent operational interaction and controlled modification of this domain. Since the fundamental definition of Biogeny involves the well-ordered, information carrying macromolecule, electron microscopy appears to be one of the most promising direct approaches to its detection. In this connection, detection of any type of ordered macromolecules or their derivatives--which can only be accomplished directly by electron optical techniques--would provide invaluable information relevant to the origin of life, giving clues to its cognogenic, biogenic, or chemogenic processes.

From these considerations stem the basic concepts of miniaturized and mobile electron microscope "stations" embodying appropriately miniaturized componentries for an integrated collection and transport of specimens, physico-chemical and physical processing of the samples remotely controlled by servo systems and coupled with a vidicon-transmitter telemetry chain. Such a miniaturized electron microscope station would not only serve as a powerful analytical tool for detection of life on Mars, but also for exobiological studies in general.

An electron microscope equipped with a miniaturized ultramicrotome incorporating a diamond knife or a diamond drill for production of ultrathin sections, which can be read off directly with the attached electron microscope, would serve all of these required functions in a practical and efficient way. As described later on in the paper, these systems would not in any way replace, but in fact ideally supplement, the contemplated automatic light microscope systems for use on a planet as proposed by J. Lederberg and his associates.

2. A supplementary paper, entitled "Potential Use of Electron Microscopy for Ultraminiaturized Information Storage and Retrieval with Electron Optical Demagnification, Combined with Direct Retrieval of Recorded Microtape to Supplement Telemetry in Exobiology" reports that the basic problem limiting the information retrieval envisaged from Mars or any other planetary mission seems to be the limit to the bits of information obtainable, of the order of about 10^9 bits of information, which is given by inherent telemetry parameters. There is also a long time interval involved. These limitations impose severe restrictions in the design of any type of system for the detection of extra-terrestrial life.

Specifically, it is proposed that all of the information obtained during the Mars missions and other extraterrestrial missions be considerably condensed by electron optical demagnification (ratio of demagnification, 1:1,000 to 1:50,000 or more). This would mean that bits of information,

greater in number by several orders of magnitude, can be imprinted directly onto reels of special ultrathin tape by demagnification electron microscopy. The ultrathin tape of silver halide is about 100 to 200 Å thick and can be mounted on suitable resistant thin tapes of rhenium, tantalum, or other refractory material. The amount of information that could be recorded in a tape reel with a total area the size of a type-written page varies from the content of a 1,000,000 volume library (each volume: 500 pages) to approximately a 10,000,000 volume library. A roll of this ultramicrotape after recording would be wound onto a bobbin of only a few cubic centimeters at most. This bobbin could be detached and provided with its own jet propulsion (plasma or other type of propulsion that is practical and long lived for such a small object) and with a radio beacon or other device to indicate and monitor its presence and trajectory. This "space courier pigeon" (in analogy to the earlier uses of microfilm transmission by pigeon courier post) would be programmed to "home", back to earth, by making use of optimized navigational techniques. Once within the reach of our earth or retrieval capabilities in outer space, these microtape capsules could be directly retrieved and read-out in space.

It is pointed out that even if manned space flights to Mars become feasible, the use of information packages of this type is just as necessary as in today's communication network. No amount of teletype, telephone, or even television, can replace the letter, the book, drawings, pictures, blueprints, etc.

This problem will become even more acute as the distances to other planets and surrounding perturbations (magnetic field, sunspots, etc.) make direct electro-magnetic radio communications very long or actually impossible. Sooner or later, we will have to develop some type of a compact, condensed, ultraminiaturized information storage and retrieval system. It will also be one of the safest ways of sampling alien environments with a minimum of cross-contamination, since a great deal of information can be recorded and transmitted without effecting actual bodily contact.

It should be noted that development of this idea on earth is already of key significance in order to cope with the critical problem of information condensation and retrieval under the conditions of the present "information explosion" in the published bibliography. With all of these considerations in mind, it is suggested that this type of approach be given serious consideration and assigned a reasonably high priority since it merits a determined and concerted effort.

III. Training Program.

A. Training has been carried out collaterally with and in addition to the various research projects. In particular, a course in Cell Ultrastructure (offered during the Spring Quarter, March - June, Tuesdays and Thursdays, 2-4PM), followed by a corresponding laboratory course (4-6PM) is conducted for students and faculty of this university as well as from other institutions, including the Illinois Institute of Technology and the University of Wisconsin. (SEE ATTACHED LECTURE SCHEDULE AND LIST OF STUDENTS ATTENDING COURSE).

B. In addition, our laboratory has served as a center for consultation and discussion on advanced techniques and as a center for short-term training sessions.

1. We have cooperated with research laboratories of other universities and organizations, such as the Argonne National Laboratory, Chicago; Stanford University, California; Brown University, Rhode Island; University of California, Berkeley; McGill University, Montreal; University of Minnesota, Minneapolis; Princeton University, New Jersey; The University of Texas, Austin; University of Pittsburgh, Pittsburgh; University of Wisconsin, Madison; and the Motorola Company, Franklin Park, Illinois.

2. The laboratory served as a center for short-term training sessions. Some of the visiting scientists, graduate students, and technicians included:

a. Dr. James Perdue, University of Wisconsin. Participating in Cell Ultrastructure Course and observing laboratory preparation techniques and research procedures.

b. Dr. Robert Bock, University of Wisconsin. Collaborative studies on Fatty-acid synthetase.

c. Dr. Elizabeth Bachmann, University of Wisconsin. Collaborative studies on enzymes.

d. Dr. Bucholz, University of Wisconsin. Collaborative studies on Fatty-acid synthetase.

e. Mr. Rod MacGregor, University of California, Berkeley. Studied preparation and instrumentation techniques of electron microscopy.

f. Dr. David Allmann, University of Wisconsin. Collaborative studies on enzymes.

g. Mr. Robert Oliver, University of Texas. Studied preparation techniques for electron microscopy.

h. Dr. John Farrant, Chemical Research Labs, Melbourne, Australia. Consultant in high resolution electron microscopy.

We are now preparing for the wide spectrum of trainees anticipated: graduates, undergraduates, post-doctorals, technicians and teachers. The training program will be coordinated with that of the proposed NASA University of Chicago Center for Science Education.

BIOPHYSICS 308 Spring 1965

CELL ULTRASTRUCTURE

Research Institutes -- Room 211 T. Th. 2-4 P.M.

March 30	History of biological ultrastructure and its unifying role in medicine and the natural sciences.
April 1	Survey of methodology with particular reference to applications in cell ultrastructure research.
April 6	Principles of structure. Crystals, liquid crystals, colloidal solutions and gels.
April 8	Elements of crystallography. Polarization microscopy and x-ray analysis.
April 13	Applications of x-ray diffraction in biological ultrastructure.
April 15	Principles of electron optics. Electron diffraction.
April 20	Introduction to electron microscopy. Concepts and methodology.
April 22	Image characteristics. Critical evaluation of preparative and instrumental limitations.
April 27	Molecular structure of proteins, lipids, and carbohydrates (I) Dr. E. van Bruggen.
April 29	Molecular structure of proteins (II): Organization of Hemocyanins. Dr. E. van Bruggen.
May 4	High resolution electron microscopy and its applications in correlative ultrastructure studies.
May 6	Molecular organization of cell membranes and their derivatives.
May 11	Correlation of structure and function in photosynthetic membranes, mitochondria, chloroplasts and other lamellar systems.
May 13	Structure of nucleic acids, polynucleotides, and nucleoproteins.
May 18	Fine structure of viruses. Bacteriophages and plant viruses.
May 20	Structure of animal viruses.
May 25	Submicroscopic organization of the cell nucleus.
May 27	Submicroscopic organization of the cell cytoplasm.
June 1	Other molecular organizations of cell components, such as [illegible]
June 3	Structure and synthesis of muscle. Correlation of structure and function.
June 8	Perception and Discussion.
June 10	Final Examination

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List of Students Taking Cell Ultrastructure Course Spring 1965:

Dr. Zelma Molnar
Department of Pathology
University of Chicago

Miss Joan Wennstrom
Department of Botany
University of Chicago

Mr. Robert Stocking
University of Chicago

Mr. Sutter A. Gardanier
Department of Pathology
University of Chicago

Miss Krystyna Langowska
Department of Biochemistry
University of Chicago

Mr. Vincent L. Morris
University of Chicago

Mr. Ronald Luftig
Department of Biophysics

Mr. L. Gage
University of Chicago

Mr. J. W. MacInnes
University of Chicago

Dr. E. F. J. van Bruggen
Visiting NIH Research Fellow
Department of Biophysics
University of Chicago

Dr. Keiji Yada
Visiting Research Associate
Department of Biophysics
University of Chicago

Miss Linda LaDeur
Department of Biophysics
University of Chicago

Miss Carol Runner
Department of Biophysics
University of Chicago

Miss Debra Meddoff
Department of Biophysics
University of Chicago

Dr. James Perdue
Institute for Enzyme Research
University of Wisconsin

Dr. J. A. Gross
Illinois Institute for Technology
Research Institute
Chicago, Illinois

Mrs. Bernadine Tooper
Illinois Institute for Technology
Research Institute
Chicago, Illinois

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IV. List of Publications for 1964-1965. (25 copies of each will be sent with this report.)

A. Fernández-Morán, H. New Approaches in Correlative Studies of Biological Ultrastructure by High Resolution Electron Microscopy, paper presented at the Celebration of the Tercentenary of the Microscope in Living Biology, the Royal Microscopical Society, Bethesda, Md., April 7-9 (1963). Published in Journal of the Royal Microscopical Society, Vol. 83, Parts 1 & 2, pp. 183-195 (1964).

B. Fernández-Morán, H.; and L. J. Reed; M. Koike; and C.R. Willms, Correlated Electron Microscopic and Biochemical Studies of a Multienzyme Complex: Pyruvate Dehydrogenase Complex of Escherichia coli. Published in Science, Vol. 145, pp. 930-932, June (1964).

C. Fernández-Morán, H. Analytical Systems for Biological Study of Mars: The role of the electron microscope and electron optical techniques in Exobiology. Paper presented at Exobiology Summer study on the Biological Exploration of Mars, Stanford University, Berkeley, California, August, 1964. Announced in the Journal of Scientific Technical Aerospace Reports by the National Aeronautics and Space Administration. Reference No. SC/NsG-441. (1964).

D. Fernández-Morán, H. and Mr. Ulys Yates. Electron Microscope--Medicine's Research Tool of Unfulfilled Promise. Published in the Journal of the American Medical Association, Vol. 189, pp. 31-33, September 28, 1964.

E. Fernández-Morán, H. Biological Systems as Formed by Water. Summation and General Discussion. Paper published in Proceedings of the New York Academy of Sciences, October 5-8, 1964.

F. Fernández-Morán, H. Electron Microscopy with High-Field Superconducting Solenoid Lenses. Published in Proceedings of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451. February (1965).

IV. List of Publications for 1964-1965 (con't.).

G. Fernández-Morán, H. Application of High-Field Superconducting Solenoid Lenses in Electron Microscopy. Paper presented at Annual Meeting of the National Academy of Sciences, April (1965). Abstract in Science , Vol. 147 (1965).

H. Fernández-Morán, H. Potential Use of Electron Microscopy for Ultraminiaturized Information Storage and Retrieval with Electron Optical Demagnification, Combined with Direct Retrieval of Recorded Microtape to Supplement Telemetry in Exobiology. Paper written to supplement previous paper written for Exobiology Study on the Biological Exploration of Mars, April (1965).

V. Reports and Publications Describing Research and Training Facilities.

A. The University of Chicago. REPORTS, Vol. 15, No. 2, Summer (1964): "Magnificent Magnification."

B. The American Medical Association. Journal of the American Medical Association, Vol. 189: 31-33, September 28, 1964, "Electron Microscope--Medicine's Research Tool of Unfulfilled Promise".

C. McGraw-Hill Yearbook of Science and Technology: Photographic Highlights Section, p. 86 Cited studies by Dr. Fernández-Morán on Pre-Cambrian Rocks of the Canadian Shield (Gunflint Chert Formation of Southern Ontario) 1965.

VI. Acknowledgements.

It is a pleasure to thank Dr. H. Stanley Bennett, Dean Division of Biological Sciences; Dr. Raymond E. Zirkle, Chairman, Department of Biophysics; and Dr. William Bloom, Department of Biophysics, University of Chicago for their valuable suggestions and whole-hearted support of our program. I express deep appreciation to Prof. S. C. Collins and Prof. F. O. Schmitt of Massachusetts Institute of Technology and Dr. W. H. Sweet of the Massachusetts General Hospital for their early interest and encouragement.

Our project is indebted to Mr. C. L. Berrington, Mr. G. Monito, Mr. A. Peuron, Mr. D. Kasun, Dr. S. Autler, Dr. J. Hulm and Mr. Fred Lins of the Cryogenics Systems Department of Westinghouse Electric Corporation for their valuable cooperation and supervision.

We are particularly indebted to Mr. R. Szara and Prof. L. Meyer of the Low Temperature Laboratory; Mr. John Hanacek and Mr. G. Gibson of the Machine Shop; and Mr. Akerhaugen, Mr. John Costa, and Mr. Helmut Krebs of the Central Development Shop for their most valuable suggestions and technical assistance.

We are, of course, greatly obliged to our own staff: Mr. L. Ouwerkerk, Dr. K. Yada, Dr. E.F.J. van Bruggen, Mr. R. Luftig, Mr. M. Ohtsuki, Mr. C. Hough, Mr. O. Winkler, Mr. H. Schilder, Miss L. LaDeur, Miss C. Runner, Mrs. K. Ryder, Mrs. S. Schmidt, Miss A. Hollinger, and Miss J. Richardson for their active participation in carrying out this project.

We wish to thank Mr. G. Olson and Mr. W. Connett of the Department of Buildings and Grounds; Mr. C. Mokstad, Asst. to Dean, Division of Physical Sciences; Mr. J. Turner, Division Budget Office; and Miss Irene Fagerstrom, Mrs. C. Morris, and Mr. J. Johnson of the Office of the Vice-President for Special Projects for their kind help.

It is also a pleasure to thank Dr. George Jacobs, Dr. Orr Reynolds, and Dr. F. Quimby of the National Aeronautics and Space Administration for their active concern and support for the project.

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List of Publications Included with this Report

(15 copies of each will be sent to the NASA Scientific and Technical Information Facility)

1. New Approaches in Correlative Studies of Biological Ultrastructure by High Resolution Electron Microscopy in Journal of the Royal Microscopical Society, Vol. 83 Parts 1 & 2, pp. 183-195 (1964).

2. Correlated Electron Microscopic and Biochemical Studies of a Multienzyme Complex: Pyruvate Dehydrogenase Complex of Escherichia coli, in Science, Vol. 145, pp. 930-932, June (1964).

3. Analytical Systems for Biological Study of Mars: The role of the electron microscope and electron optical techniques in Exobiology, in Journal of Scientific Technical Aerospace Reports by the National Aeronautics and Space Administration. Reference No. SC/NsG-441. (1964).

4. Electron Microscope--Medicine's Research Tool of Unfulfilled Promise, in the Journal of the American Medical Association, Vol. 189, pp. 31-33, September 28, 1964.

5. Biological Systems as Formed by Water. Summation and and General Discussion, in Proceedings of the New York Academy of Sciences, October 5-8, 1964.

6. Electron Microscopy with High-Field Superconducting Solenoid Lenses, in Proceedings of the National Academy of Sciences, Vol. 53, No. 2, pp. 445-451, February, 1965.

7. Application of High-Field Superconducting Solenoid Lenses in Electron Microscopy. Abstract in Science, Vol. 147, p. 665, May, 1965.

8. Potential Use of Electron Microscopy for Ultraminiaturized Information Storage and Retrieval with Electron Optical Demagnification, Combined with Direct Retrieval of Recorded Microtape to Supplement Telemetry in Exobiology.

9. Magnificent Magnification, in The University of Chicago REPORTS, Vol. 15, No. 2, Summer 1964.

10. Electron Microscope--Medicine's Research Tool of Unfulfilled Promise, in Journal of the American Medical Association, Vol. 189: 31-33, September 28, 1964.

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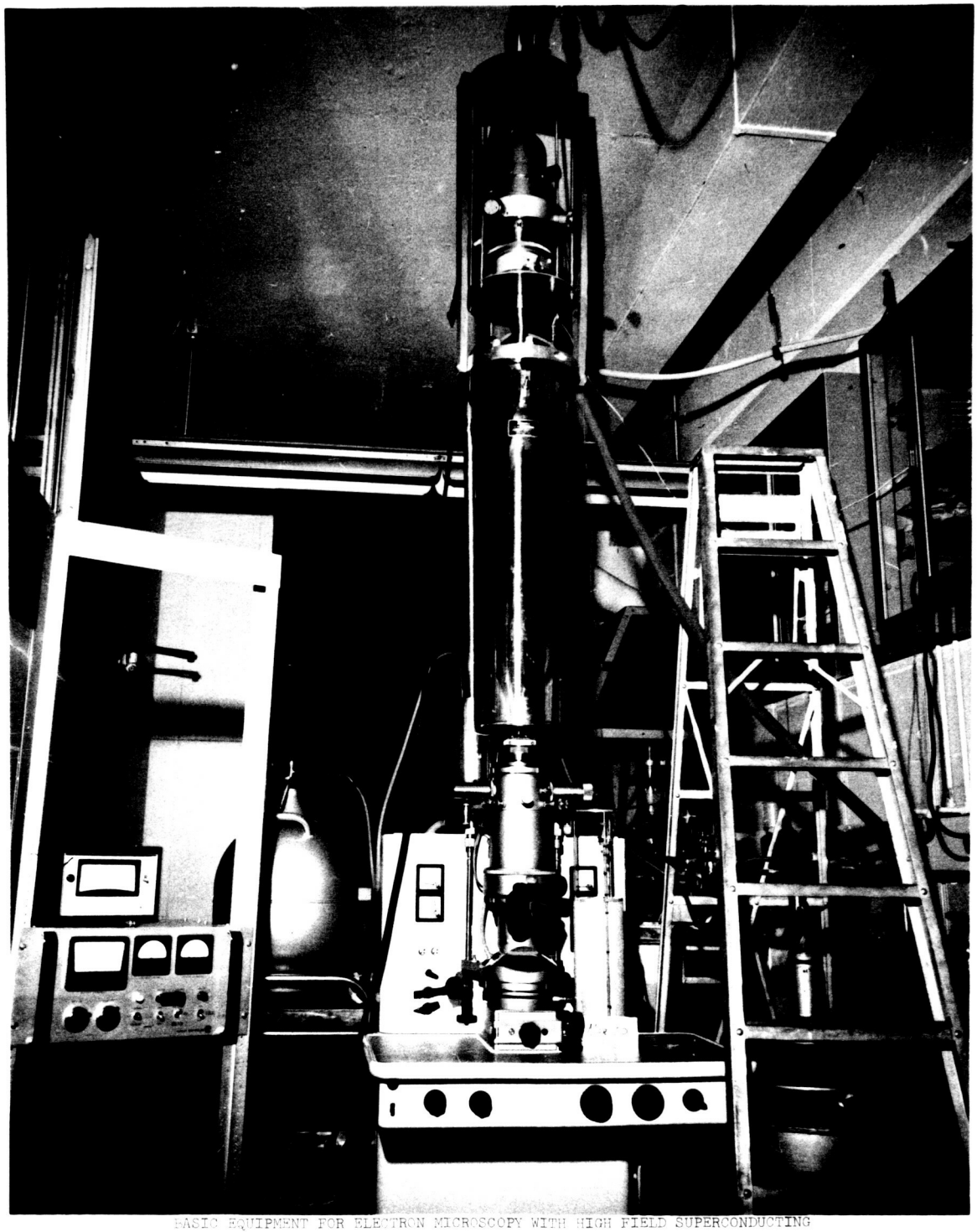
The following figures illustrate work in electron microscopy with high-field superconducting solenoid lenses and other research work in progress.



BASIC EQUIPMENT FOR ELECTRON MICROSCOPY WITH HIGH FIELD SUPERCONDUCTING LENSES, COMPRISING AIR CORE LIQUID HELIUM DEWAR WITH SUPERCONDUCTING SOLENOID(operating at 32,200 gauss in persistent current mode),AND INSERTED ELECTRON MICROSCOPE COLUMN. THE ELECTRON GUN WITH POINTED FILAMENT SOURCE OPERATES WITH HIGHLY REGULATED ACCELERATING POTENTIAL OF 50 kV AND IS MOUNTED ON COMMERCIAL ELECTRON MICROSCOPE BASE(Siemens Elmiskop II) TO FACILITATE ROUTINE OPERATION AND DIRECT PHOTOGRAPHIC RECORDING ON KODAK HIGH RESOLUTION 70 mm FILM.

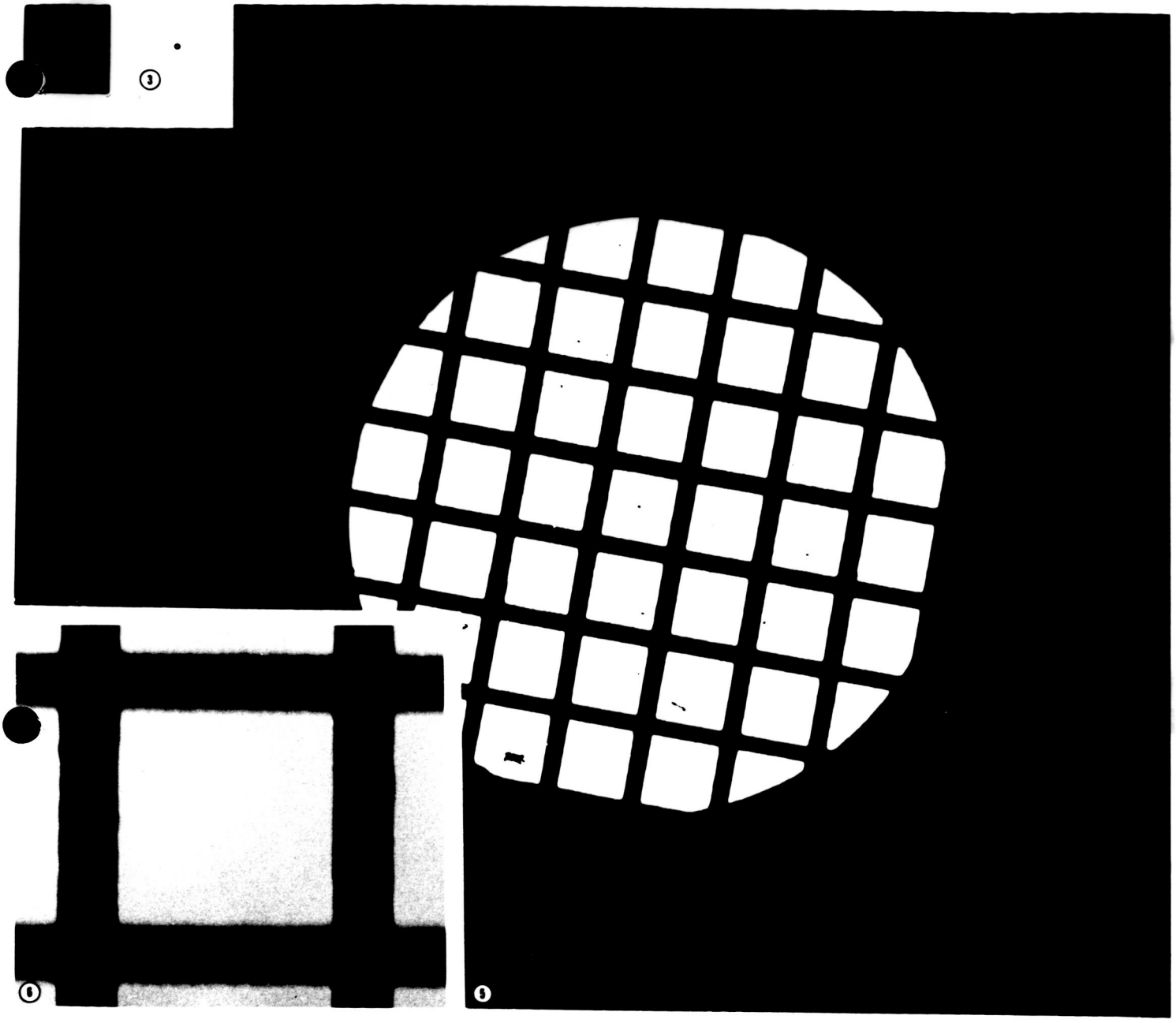


FIG. 3: MICROGRAPH OF COPPER SPECIMEN GRID (200 MESH). NATURAL SIZE.

FIG. 4: SAME GRID PHOTOGRAPHED DIRECTLY IN CRYO-ELECTRON MICROSCOPE (6kV) WITHOUT MAGNETIC FIELD. MAGNIFICATION: 3 X.

FIG. 5: ELECTRON MICROGRAPH OF SPECIMEN GRID RECORDED DIRECTLY ON PHOTOGRAPHIC PLATE (ILFORD HIGH RESOLUTION PLATE) WITH HIGH FIELD SUPERCONDUCTING LENS (22,200 gauss in PERSISTENT CURRENT MODE) IN CRYO-ELECTRON MICROSCOPE WITHOUT POLE PIECES; 6 kV ACCELERATING POTENTIAL. MAGNIFICATION: 260 X.

FIG. 6: SPECIMEN GRID SECTION FROM FIG. 5. MAGNIFICATION: 850 X.

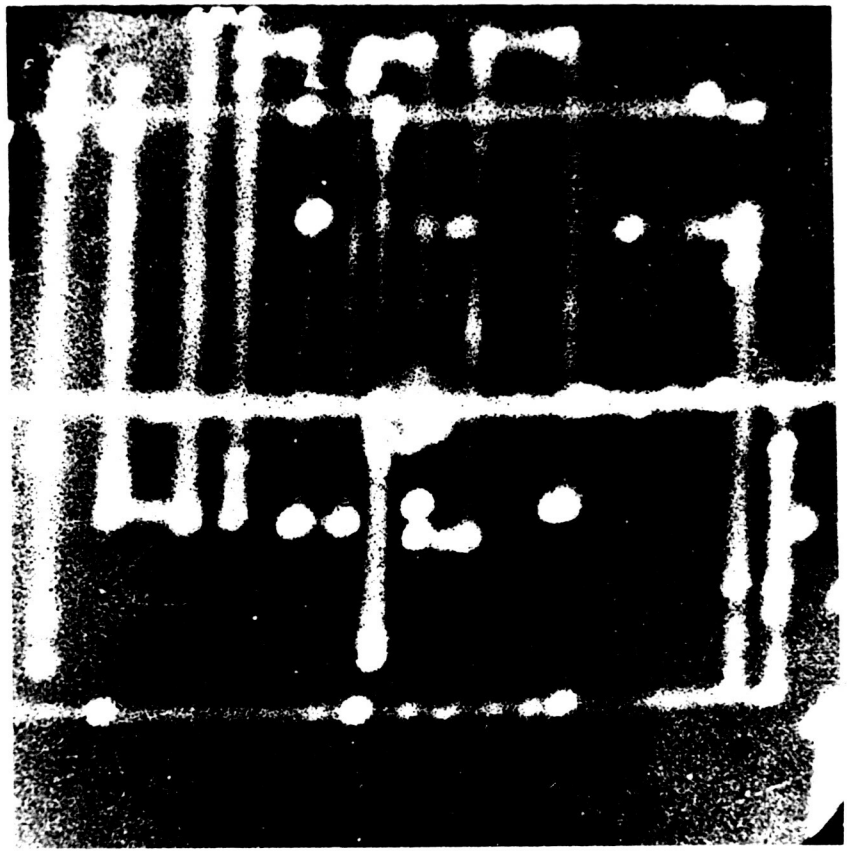
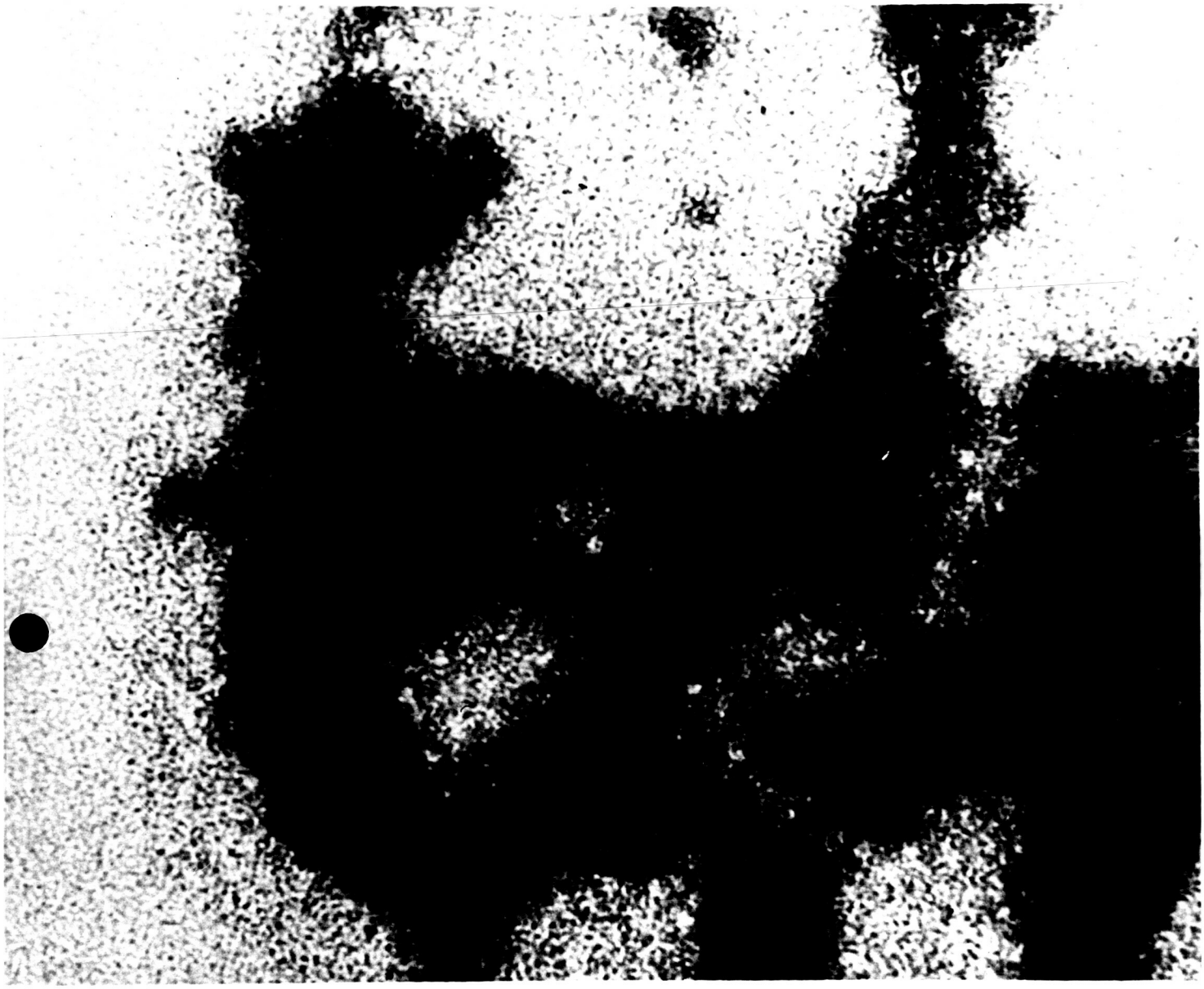


Fig. 2: ELECTRON MICROGRAPH OF ULTRAMINIATURIZED CIRCUIT PATTERNS PRODUCED BY PHOTOENGRAVING WITH ELECTRON MICROBEAM PROBES (500 to 1000 Å diameter) ON SPECIAL ULTRAFINE PHOTOGRAPHIC FILM. X 10,000.

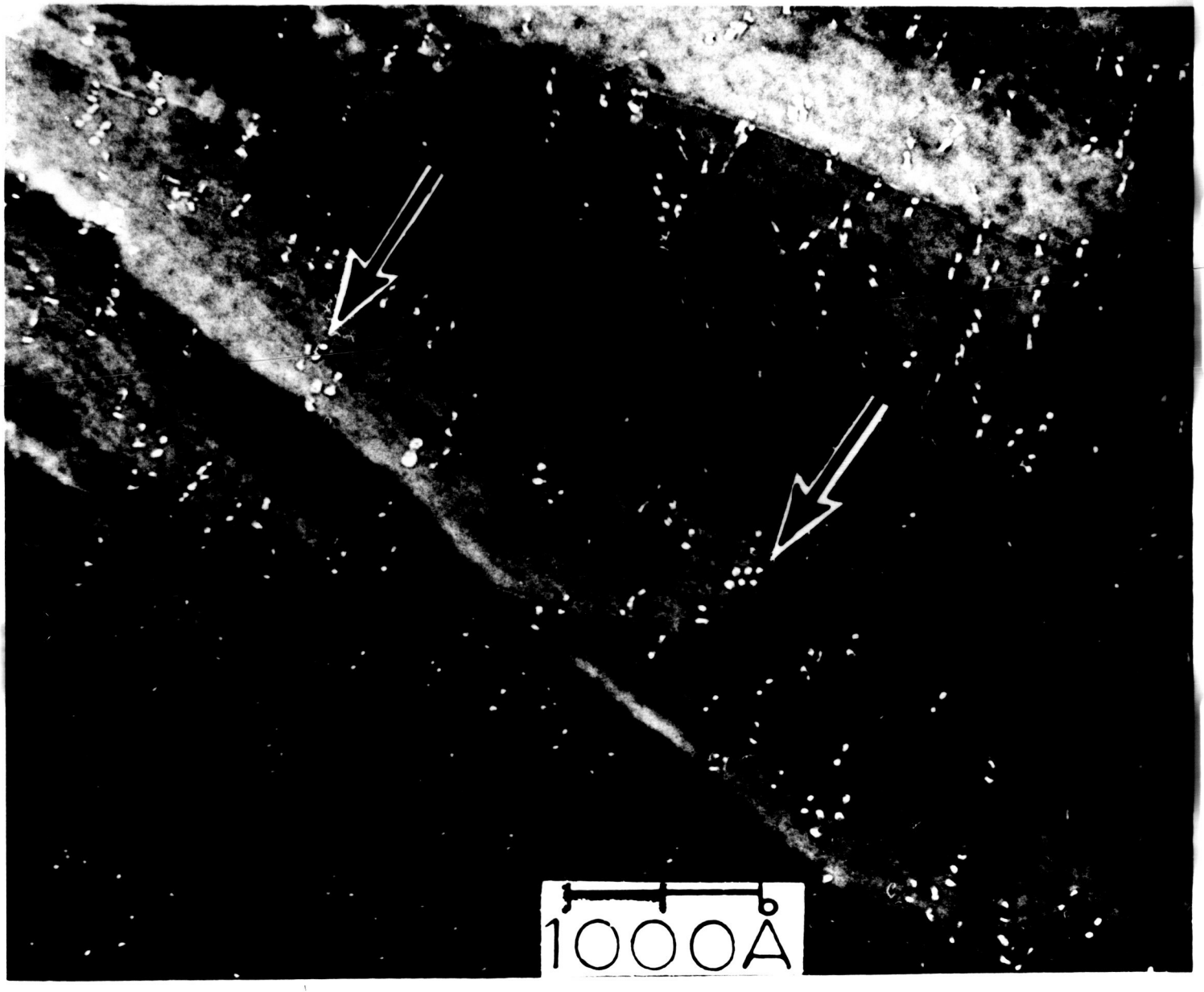
Fig. 3: MINIATURIZED LETTERS (of less than 1 micron) ENGRAVED ON THIN COLLODION FILM USING ELECTRON MICROBEAM PROBES PRODUCED BY DEMAGNIFICATION IN AN ELECTRON MICROSCOPE. (G. Möllenstedt and R. Speidel, Physik. Bl. 16,192,1960)



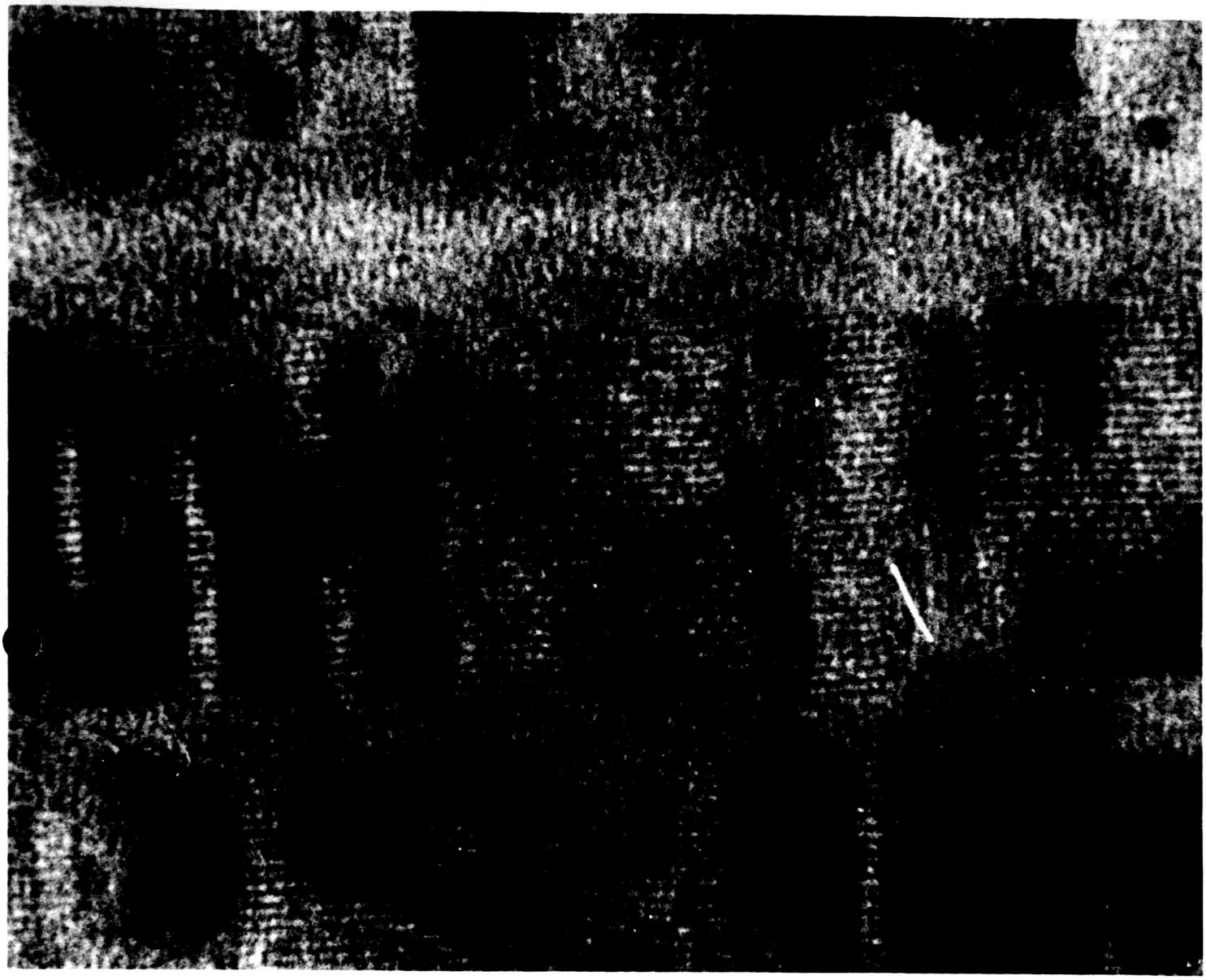
18S PARTICLES FROM METHIONINE-STARVED E. coli NEGATIVELY STAINED BY CROSS-SHRAYING WITH URANYL ACETATE. THESE ARE POSSIBLE PRECURSORS OF THE 30S RIBOSOME SUBUNIT. PREPARATION BY R. HASELKORN AND H. MANOR. ELECTRON MICROSCOPY BY H. FERNANDEZ-MORAN.

ELECTRON OPTICAL MAGNIFICATION: 40,000 X.

TOTAL MAGNIFICATION: 1,700,000 X



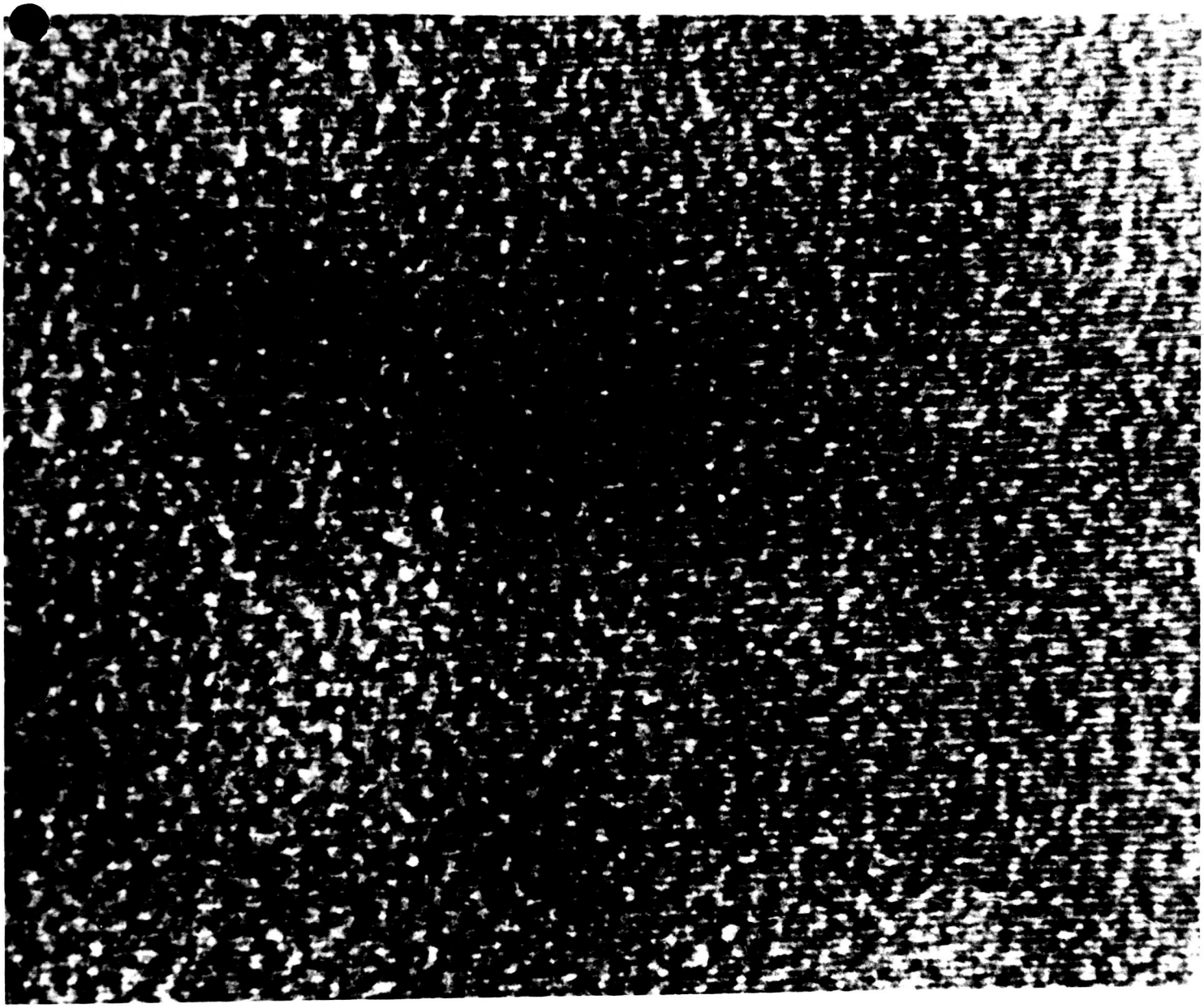
ELECTRON MICROGRAPH OF SPECIAL PENETRATED FILMS OF SINGLE-CRYSTAL MICA (thickness ca. 100Å) WITH HOLES OF 50 to 100Å DIAMETER. THESE HOLES ARE PRODUCED BY FISSION FRAGMENTS IN IRRADIATED MICA, WHICH IS SUBSEQUENTLY ETCHED AND PREPARED ACCORDING TO THE METHOD DESCRIBED BY R. FERNANDEZ-MORAN (J. Appl. Phys. 31, 1960, 1960). SINGLE-CRYSTAL FILMS WITH SUBMICROSCOPIC HOLES OF THIS TYPE ARE VERY SUITABLE FOR HIGH RESOLUTION ELECTRON MICROSCOPY OF BIOLOGICAL SYSTEMS.
ELECTRON INITIAL MAGNIFICATION: 600,000 X.



HIGH RESOLUTION ELECTRON MICROGRAPH OF K_2PtCl_4 SHOWING LATTICE SPACING OF (100) PLANES. SPACING RESOLVED IS 6.70 Å BY DIRECT (AXIAL) ILLUMINATION METHOD, IN CONTRAST TO THE TILTED ILLUMINATION METHOD USED BY MENTER, KUMODA ET AL AND DOWELL. RECORDED WITH HITACHI HU-8 BY DR. KEIJI YADA USING LIQUID NITROGEN ANTICONTAMINATION DEVICE AND COLD STAGE AT -150°C.

ELECTRON OPTICAL MAGNIFICATION: 200,000 X.

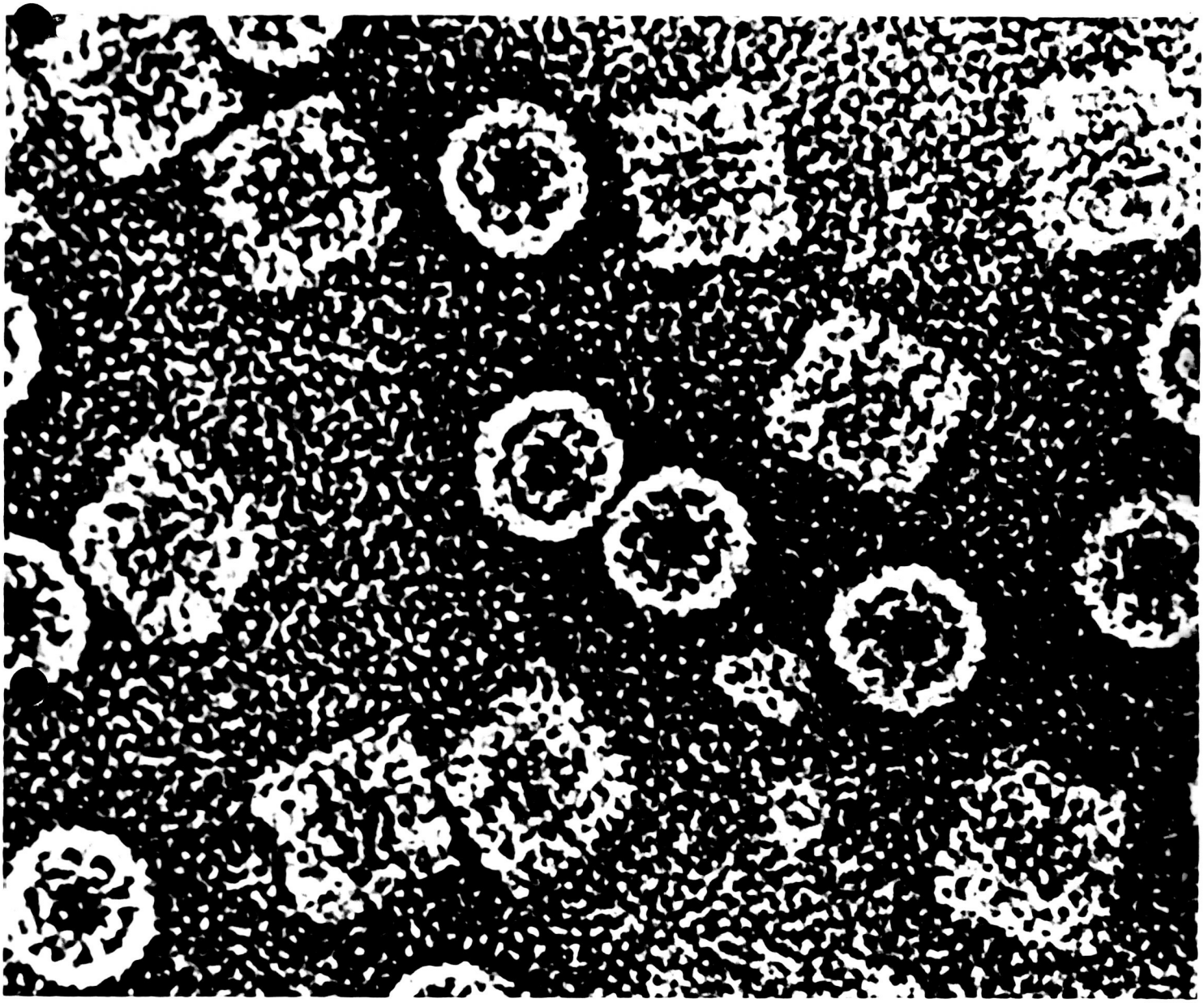
TOTAL MAGNIFICATION:



HIGH RESOLUTION ELECTRON MICROGRAPH OF PYROPHYLLITE CRYSTAL SHOWING LATTICE SPACING OF (020) PLANES.
SPACING RESOLVED IS 4.47 Å BY DIRECT (AXIAL) ILLUMINATION METHOD, IN CONTRAST TO THE TILTED
ILLUMINATION USED BY MENTER, AOMODA ET AL AND DOWELL. RECORDED WITH HITACHI HU-8 BY DR. KEIJI YADA
USING LIQUID NITROGEN ANTICONTAMINATION DEVICE AND COLD STAGE AT -150°C.
ELECTRON OPTICAL MAGNIFICATION: [illegible] X.
TOTAL MAGNIFICATION: [illegible] X.

HIGH RESOLUTION ELECTRON MICROGRAPH OF NaCl CRYSTALS EVAPORATED ONTO CARBON COATING ON (100) PLANES.
IMAGING RESOLVED TO 2.82 Å BY OBLIQUE (AXIAL) ILLUMINATION MODIFIED BY USE OF AXIAL TANTALUM
POINTED FILAMENT IN THE HANAU EM9A. RECORDED WITH HITACHI HU-11A ELECTRON MICROSCOPE WITH
CLASSIC MICROSCOPE AREA EXAMINATION DEVICE BY DR. KRAIG YADA.
ELECTRON OPTICAL MAGNIFICATION: 1.4 x 10^5 X.
TOTAL MAGNIFICATION: ~1.4 x 10^6 X.

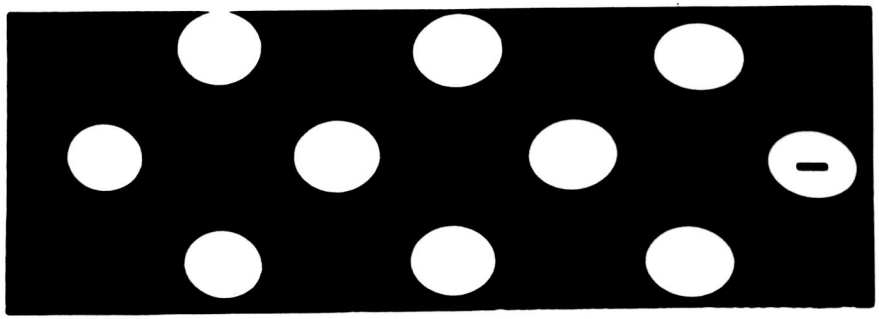
HIGH RESOLUTION ELECTRON MICROGRAPH OF A U.S. MINERAL ASBESTOS FIBRIL OF CROCIDOLITE SHOWING 47 (.94) PLANES.
SHADING RESOLVED TO 2.72 Å BY DIRECT (ANCAS) ILLUMINATION BY METHOD USING SPECIAL TANTALUM
POINTED FILAMENT OF THE MORAN TYPE. REC'RDED WITH HITACHI H-7A ELECTRON MICROSCOPE WITH
LIQUID NITROGEN ANTI-CONTAMINATION DEVICE ON SEM, ANCAS NASA.
ELECTRON OPTICAL MAGNIFICATION: 1 X, XXX X.
TOTAL MAGNIFICATION: X,X X,XXX X.



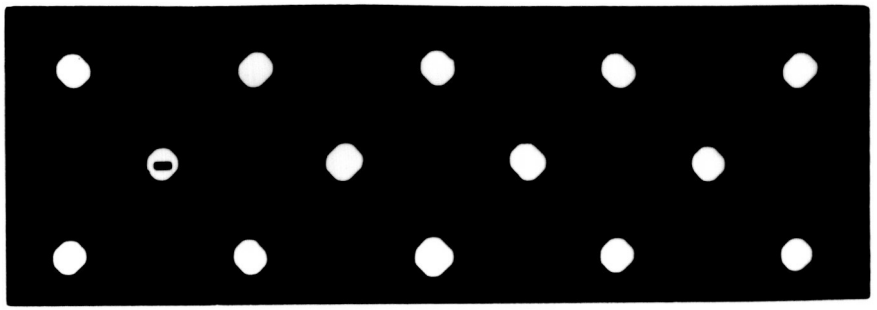
HELIX POMATIA (= ROMAN SNAIL) HEMOCYANIN MOLECULES (SEDIMENTATION CONSTANT 105S). NEGATIVELY STAINED WITH URANYL EDTA. HEMOCYANINS ARE PROTEINS FREELY DISSOLVED IN THE BLOOD OF SEVERAL INVERTEBRATES BELONGING TO THE PHYLUMS MOLLUSCA AND ARTHROPODA. THEIR FUNCTION IS THE TRANSPORT OF OXYGEN. THEIR SIZE AND STRUCTURE VARY DEPENDING ON THE BIOLOGICAL ORIGIN. THE CIRCLES AND SQUARES OBSERVED ON THE ELECTRON MICROGRAPHS ARE THE PROJECTIONS OF A CYLINDRICAL TYPE OF MOLECULES. THESE CYLINDERS HAVE A FIVE-FOLD SYMMETRY AND ARE BUILT FROM SIX PARALLEL LAYERS OF SUBUNITS. PREPARATION BY DR. E. van BRUGGEN.

ELECTRON OPTICAL MAGNIFICATION: 40,000 X.

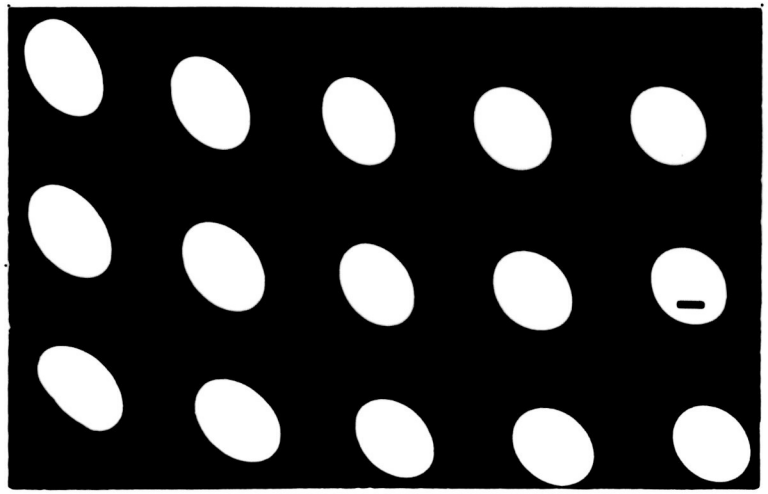
TOTAL MAGNIFICATION: 1,700,000 X



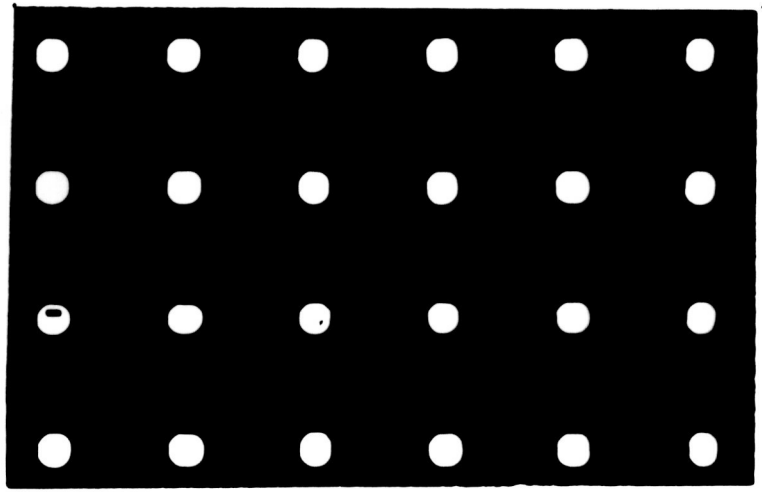
S ELECTRON MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE ;NO POLE PIECE; 50 kV. ORIGINAL MAGNIFICATION:X 400



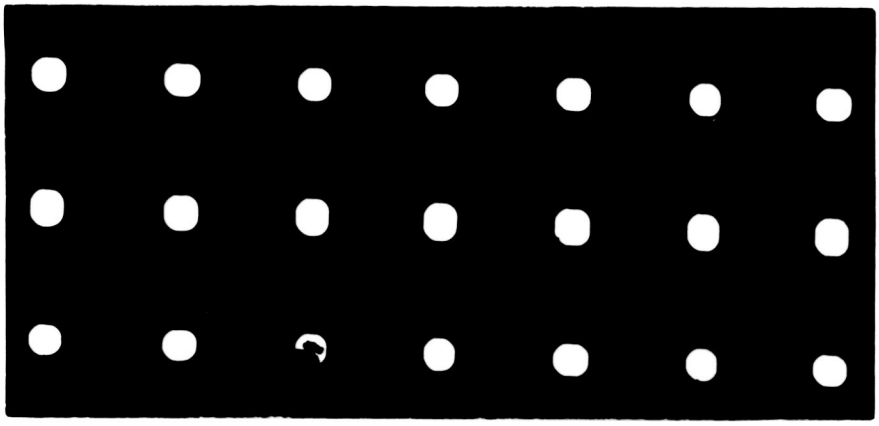
N CONTROL MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL MAGNIFICATION:X 220



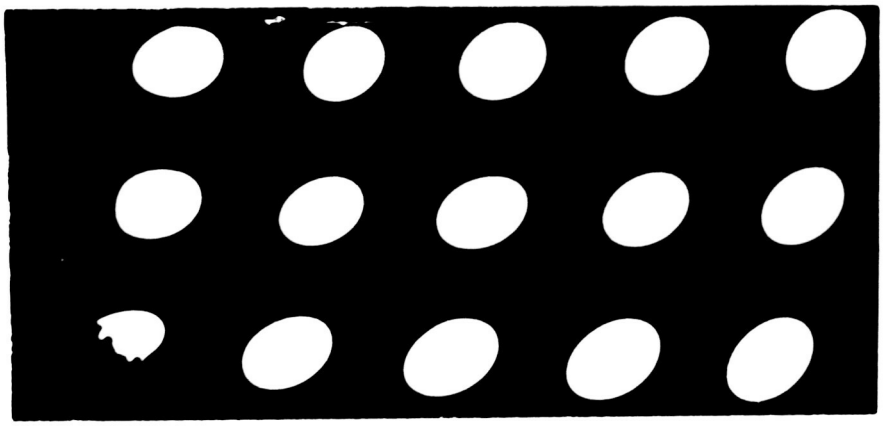
S ELECTRON MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS: PERSISTENT CURRENT MODE; NO POLE PIECE; 50 kV. ORIGINAL MAGNIFICATION: X 400.



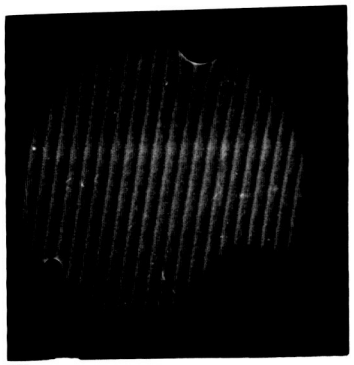
N CONTROL MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE USING OBJECTIVE POLE PIECE. ORIGINAL MAGNIFICATION: X 220.



A CONTROL MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL MAGNIFICATION: X 220



B ELECTRON MICROGRAPH OF 2000 MESH SPECIMEN GRID RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; NO POLE PIECE; 50 kV. ORIGINAL MAGNIFICATION: X 400



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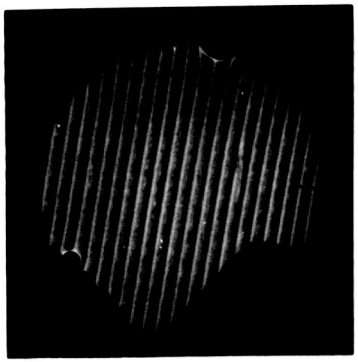

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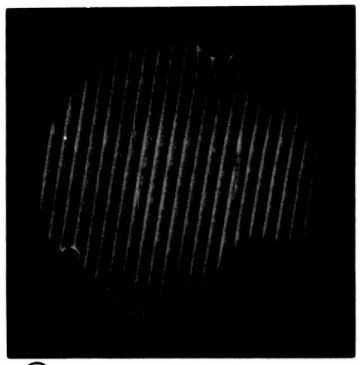

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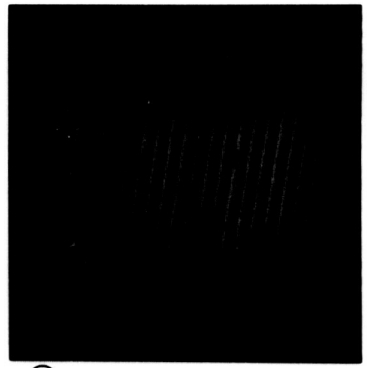

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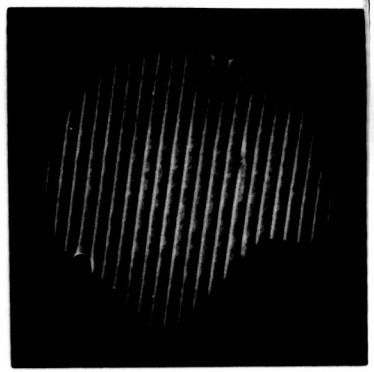
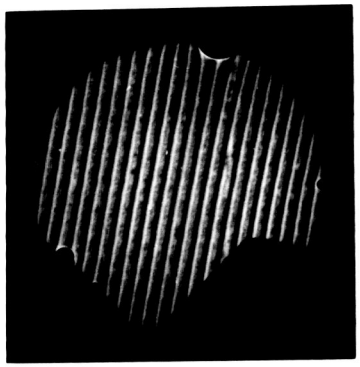


5

ELECTRON MICROGRAPHS OF REPLICA OF 28,800 lines per inch DIFFRACTION GRATING RECORDED UNDER SAME CONTROLLED CONDITIONS WITH HIGH FIELD SUPERCONDUCTING LENS OPERATING IN PERSISTENT CURRENT MODE CONTINUOUSLY OVER A TOTAL PERIOD OF 12 HOURS. MICROGRAPHS WERE RECORDED DIRECTLY ON HIGH RESOLUTION FILM AT 5 to 15 MINUTE INTERVALS IN CRYO-ELECTRON MICROSCOPE AT 50 KV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 5,000.



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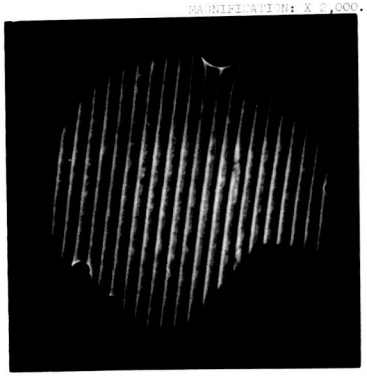

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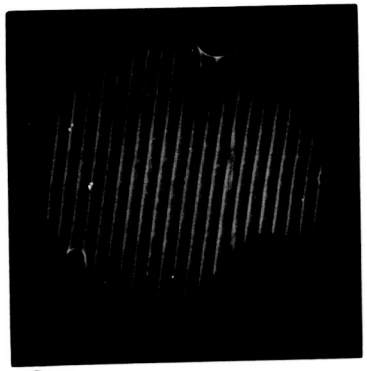

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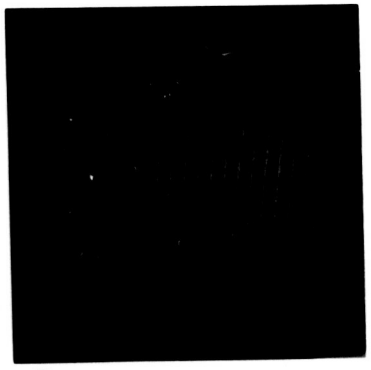

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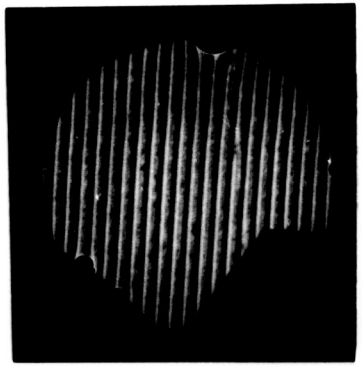
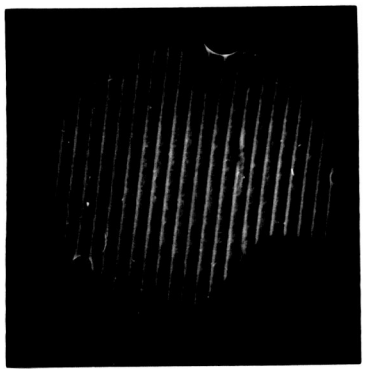


10

ELECTRON MICROGRAPHS OF REPLICA OF 28,800 lines per inch DIFFRACTION GRATING RECORDED UNDER SAME CONTROLLED CONDITIONS WITH HIGH FIELD SUPERCONDUCTING LENS OPERATING IN PERSISTENT CURRENT MODE CONTINUOUSLY OVER A TOTAL PERIOD OF 12 HOURS. MICROGRAPHS WERE RECORDED DIRECTLY ON HIGH RESOLUTION FILM AT 5 to 15 MINUTE INTERVALS IN CRYO-ELECTRON MICROSCOPE AT 50 KV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 5,000.



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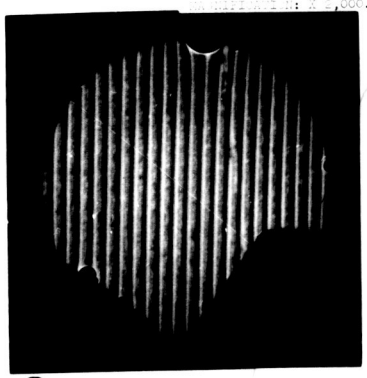

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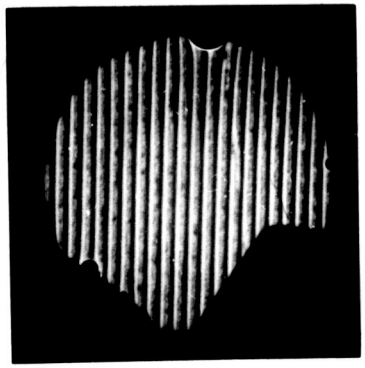

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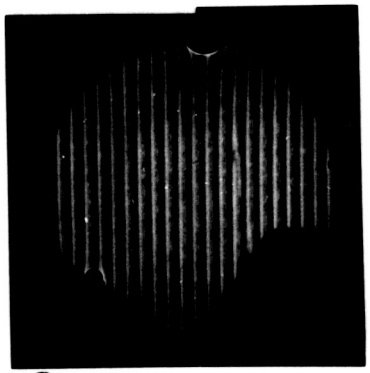

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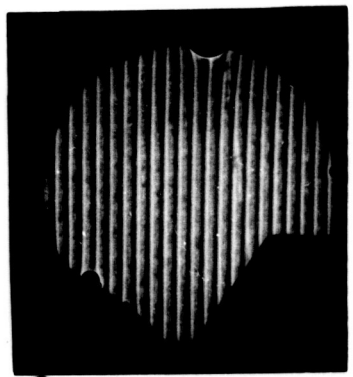

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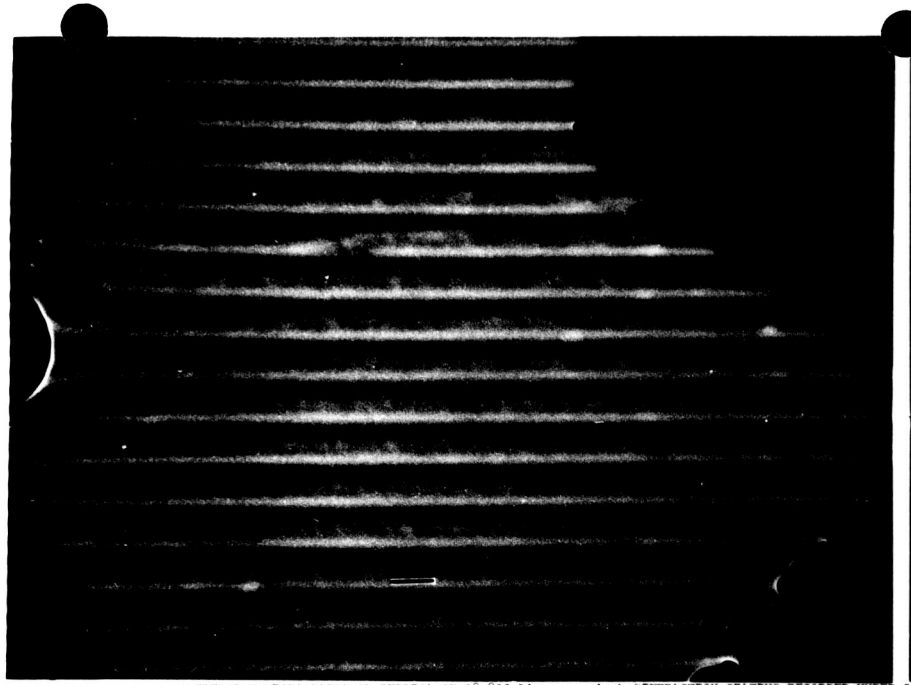
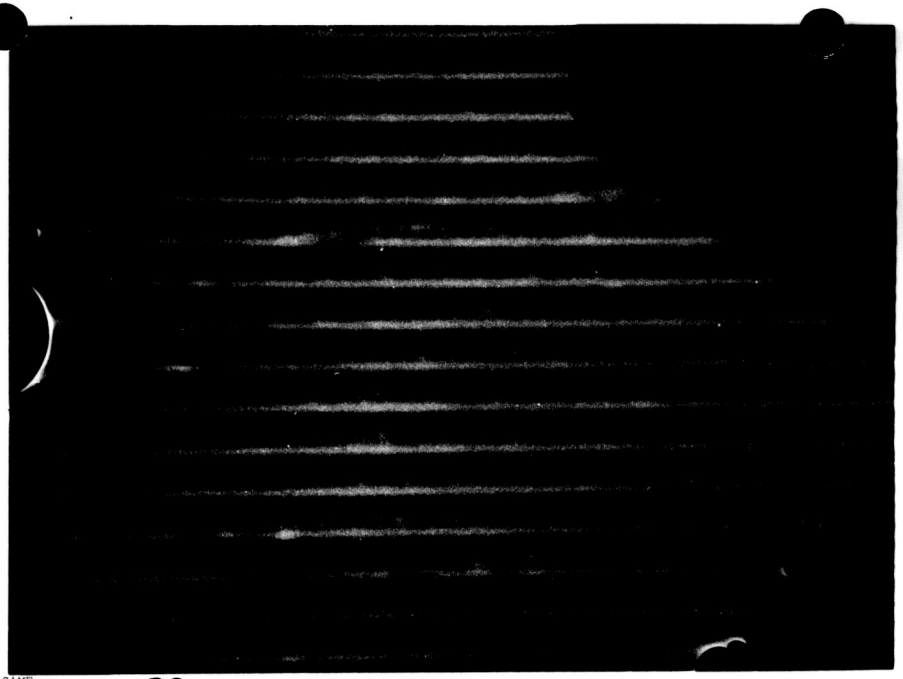
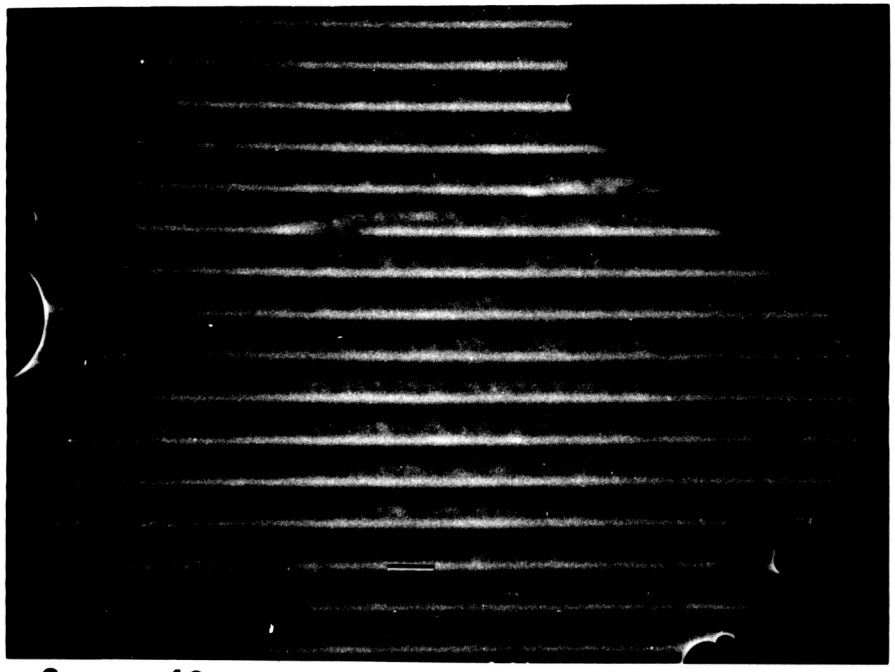


S 33

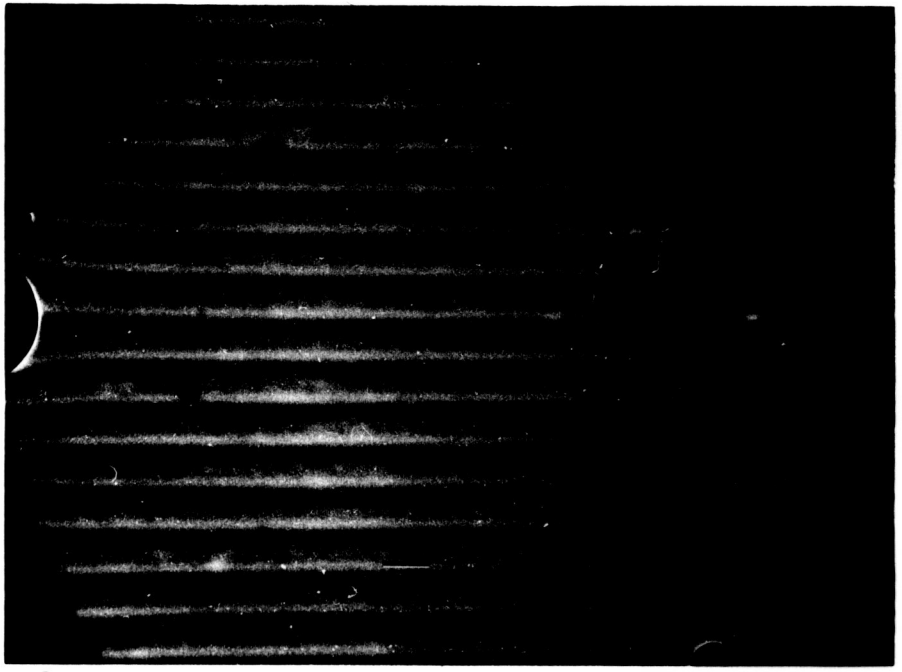


S 38

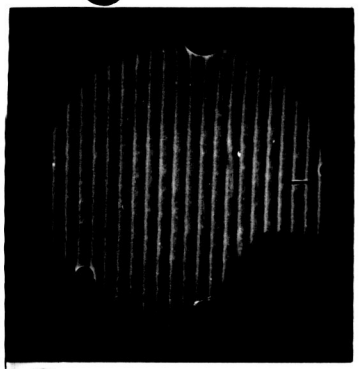
ELECTRON MICROGRAPHS OF REPLICA OF 28,800 lines per inch DIFFRACTION GRATING RECORDED UNDER SAME CONTROLLED CONDITIONS WITH HIGH FIELD SUPERCONDUCTING LENS OPERATING IN PERSISTENT CURRENT MODE CONTINUOUSLY OVER A TOTAL PERIOD OF 12 HOURS. MICROGRAPHS WERE RECORDED DIRECTLY ON HIGH RESOLUTION FILM AT 5 to 15 MINUTE INTERVALS IN CRYO-ELECTRON MICROSCOPE AT 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 8,000. TOTAL MAGNIFICATION: X 15,000.



S 40



S 69



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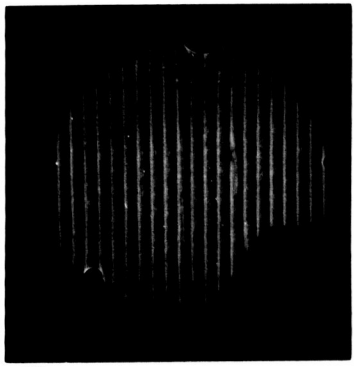

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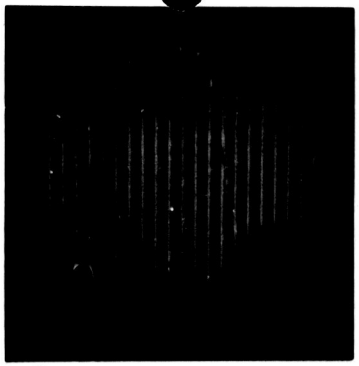

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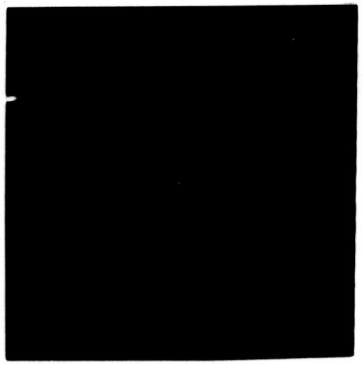

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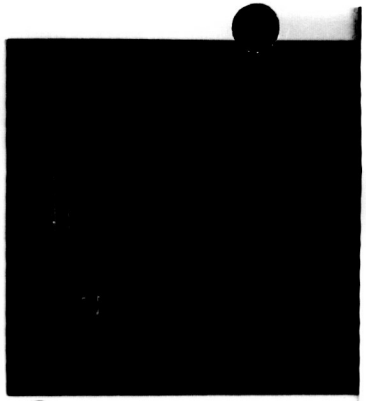
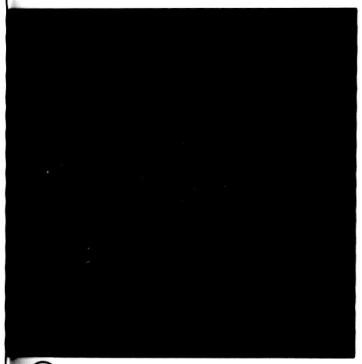


20

ELECTRON MICROGRAPHS OF REPLICA OF 28,800 lines per inch DIFFRACTION GRATING RECORDED UNDER SAME CONTROLLED CONDITIONS WITH HIGH FIELD SUPERCONDUCTING LENS OPERATING IN PERSISTENT CURRENT MODE CONTINUOUSLY OVER A TOTAL PERIOD OF 10 HOURS. MICROGRAPHS WERE RECORDED DIRECTLY ON HIGH RESOLUTION FILM AT 5 to 15 MINUTE INTERVALS IN CRYO-ELECTRON MICROSCOPE AT 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 2,000.



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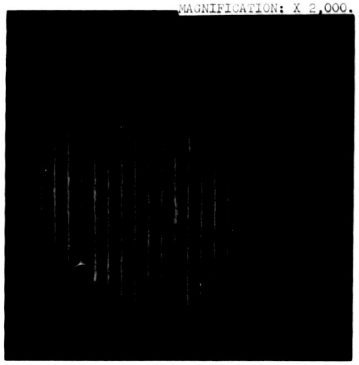

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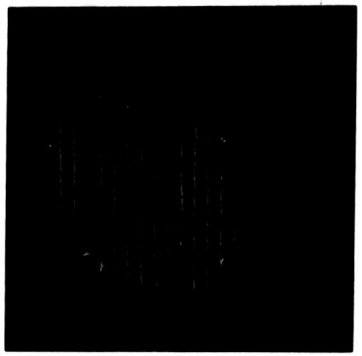

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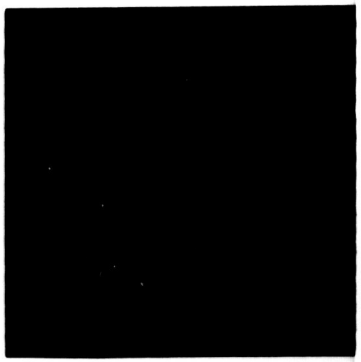
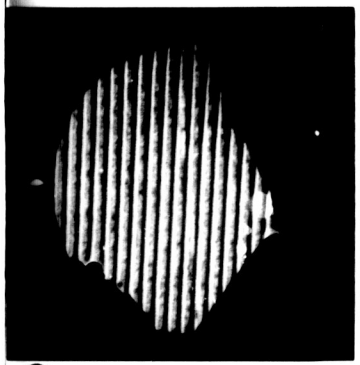


25

ELECTRON MICROGRAPHS OF REPLICA OF 28,800 lines per inch DIFFRACTION GRATING RECORDED UNDER SAME CONTROLLED CONDITIONS WITH HIGH FIELD SUPERCONDUCTING LENS OPERATING IN PERSISTENT CURRENT MODE CONTINUOUSLY OVER A TOTAL PERIOD OF 10 HOURS. MICROGRAPHS WERE RECORDED DIRECTLY ON HIGH RESOLUTION FILM AT 5 to 15 MINUTE INTERVALS IN CRYO-ELECTRON MICROSCOPE AT 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 2,000.



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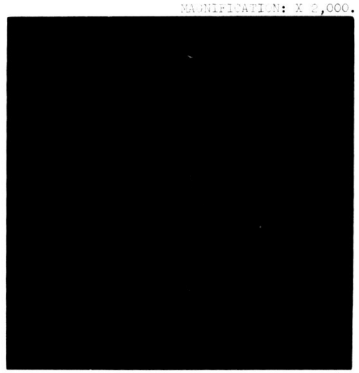

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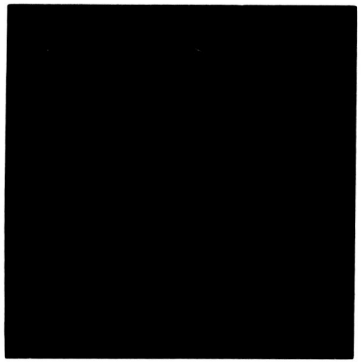

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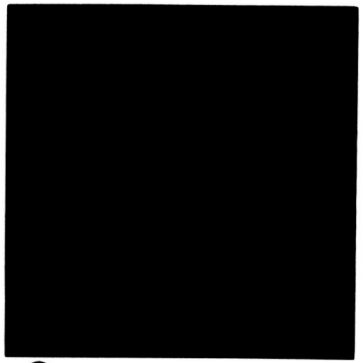

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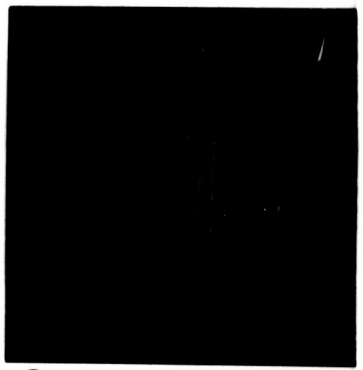

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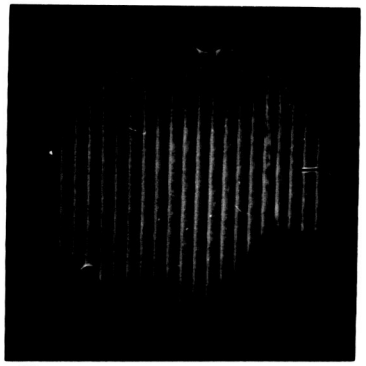

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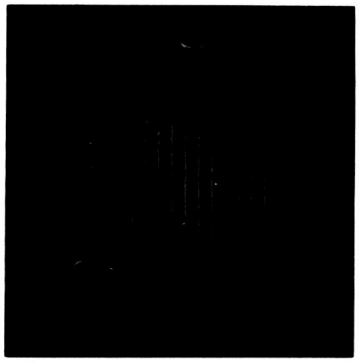

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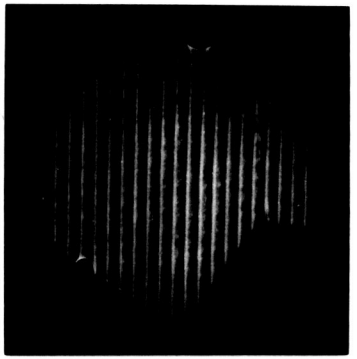

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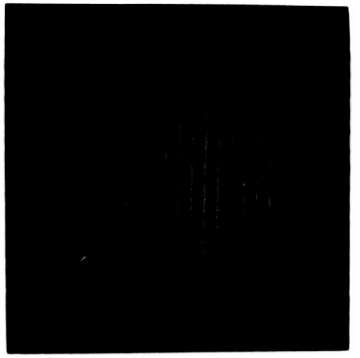

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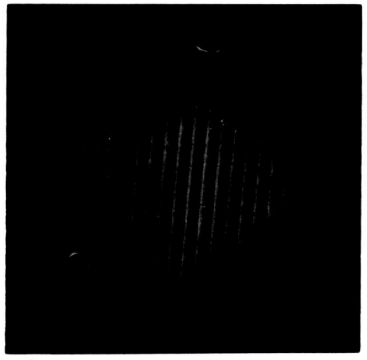

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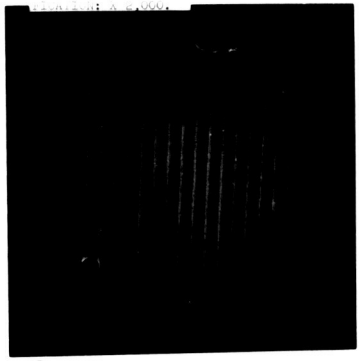

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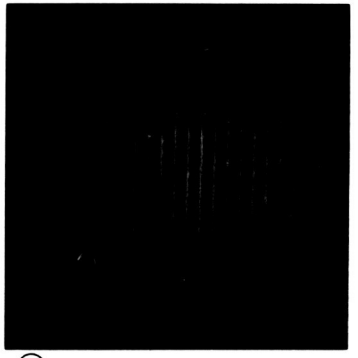

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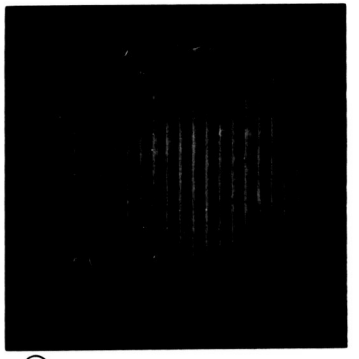

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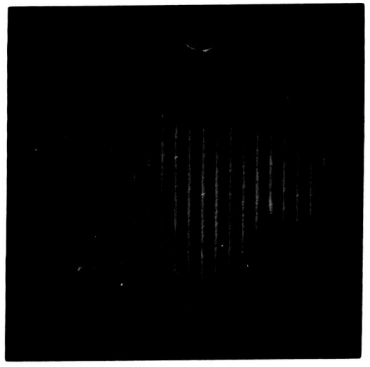

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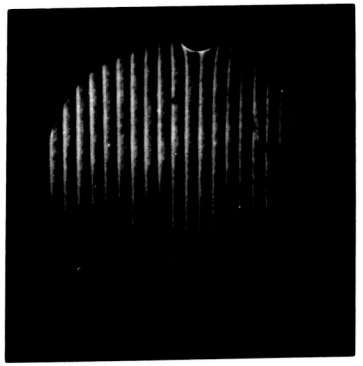

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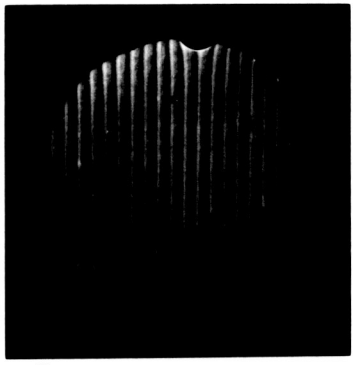

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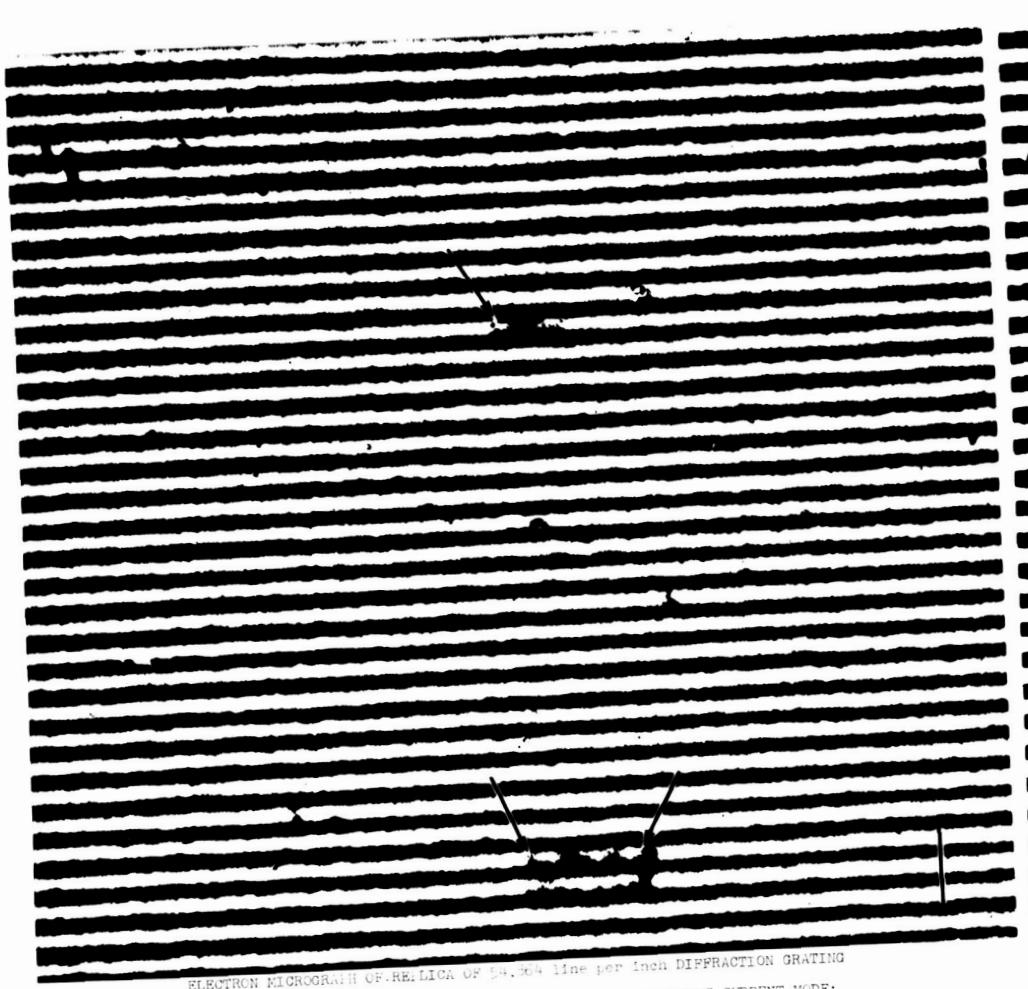
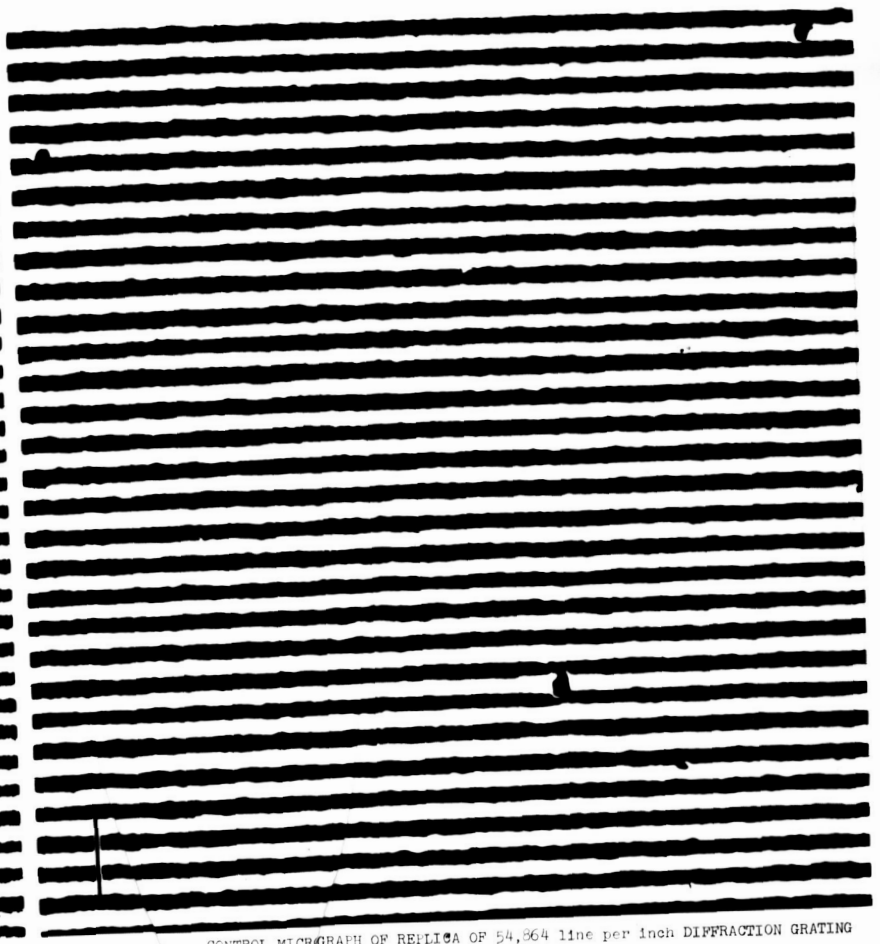


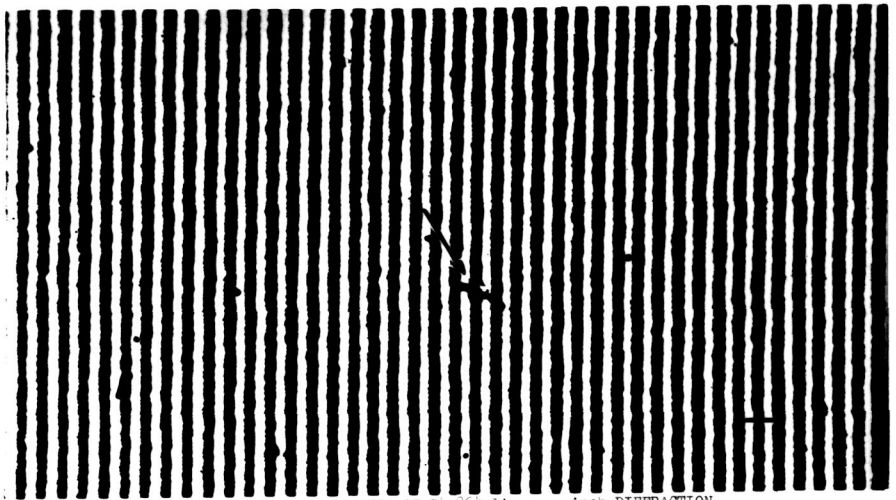
S

ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 10 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 25,000



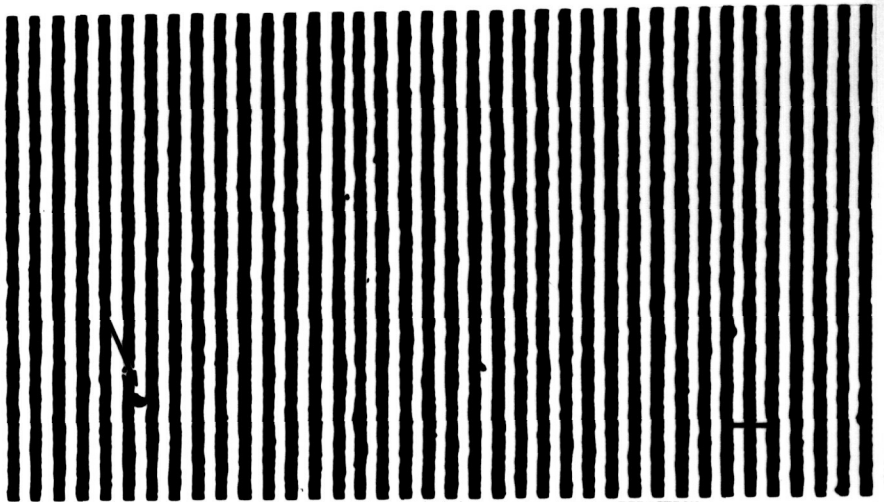
N

CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 25,000



S

ELECTRON MICROGRAPH OF REPLICA OF 54,364 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 15,000



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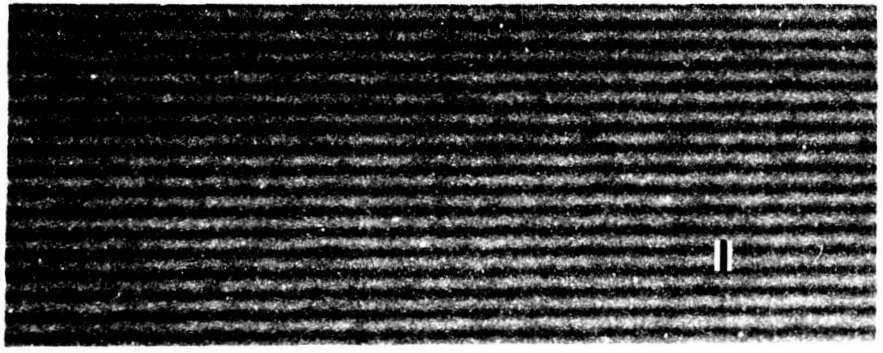
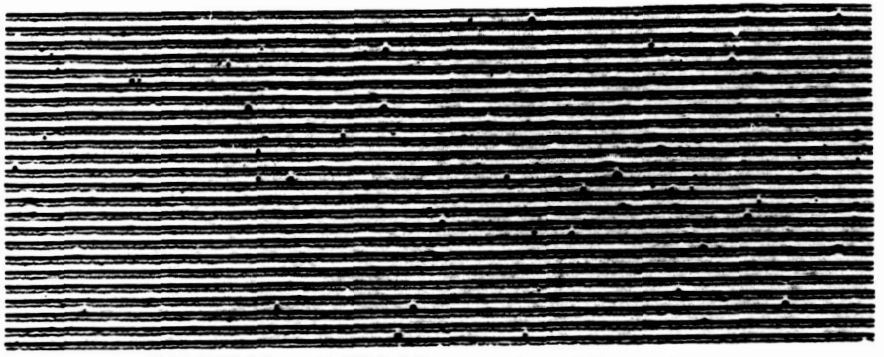
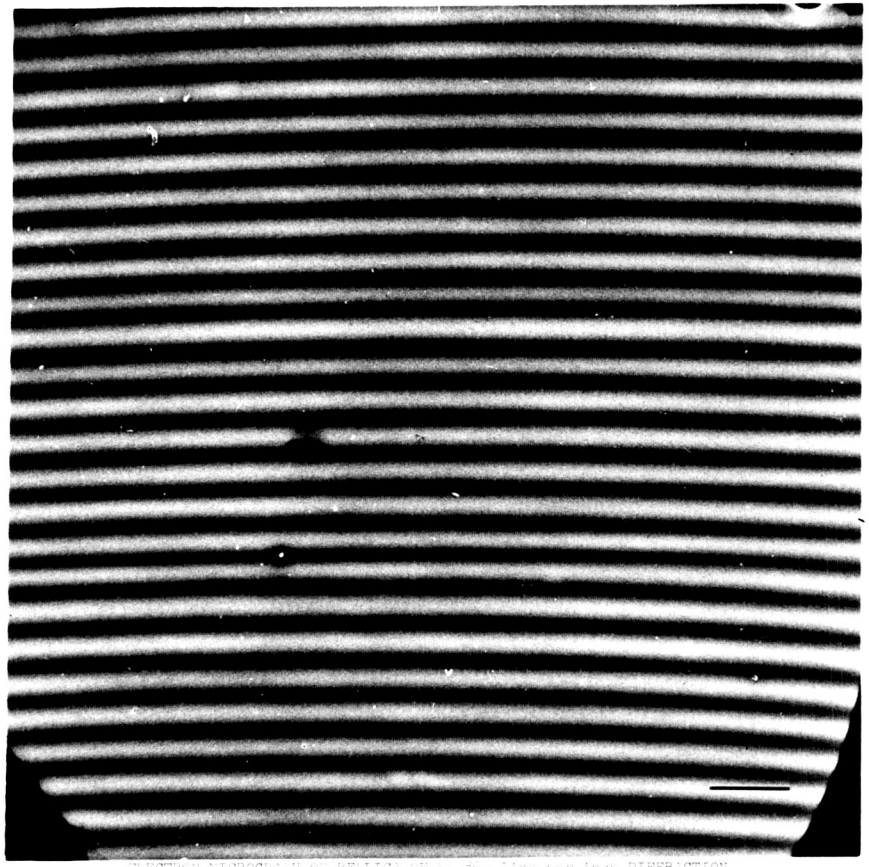
CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X, 15,000



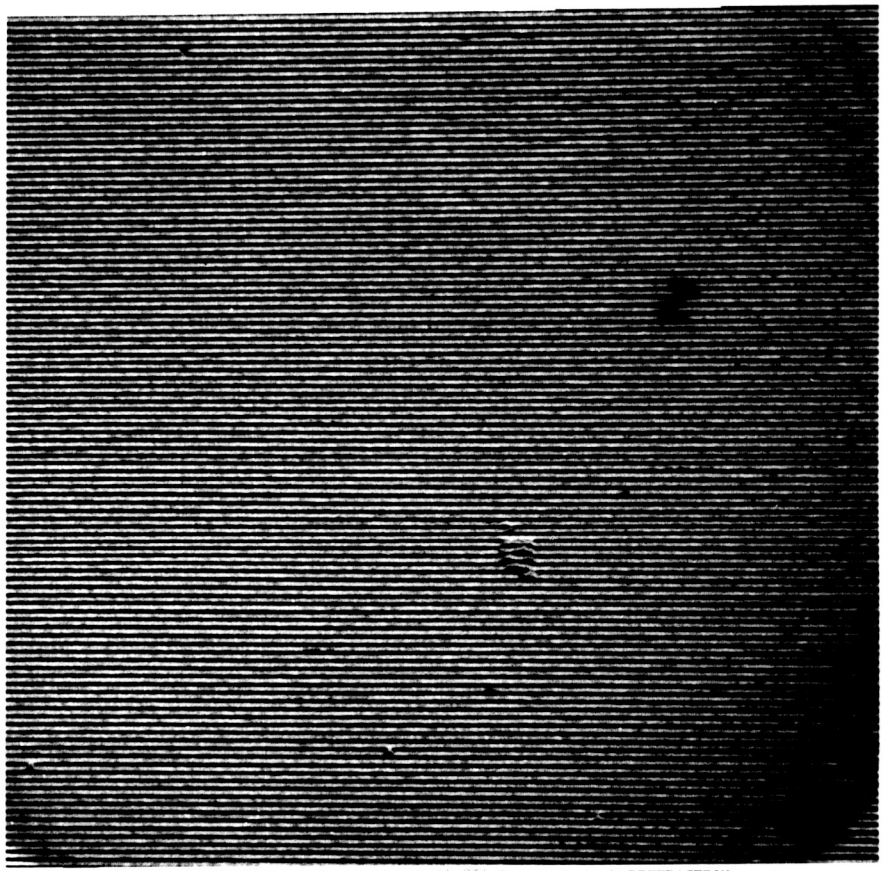
S ELECTRON MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; NO POLE PIECE; 50 kV. ORIGINAL MAGNIFICATION: X 350



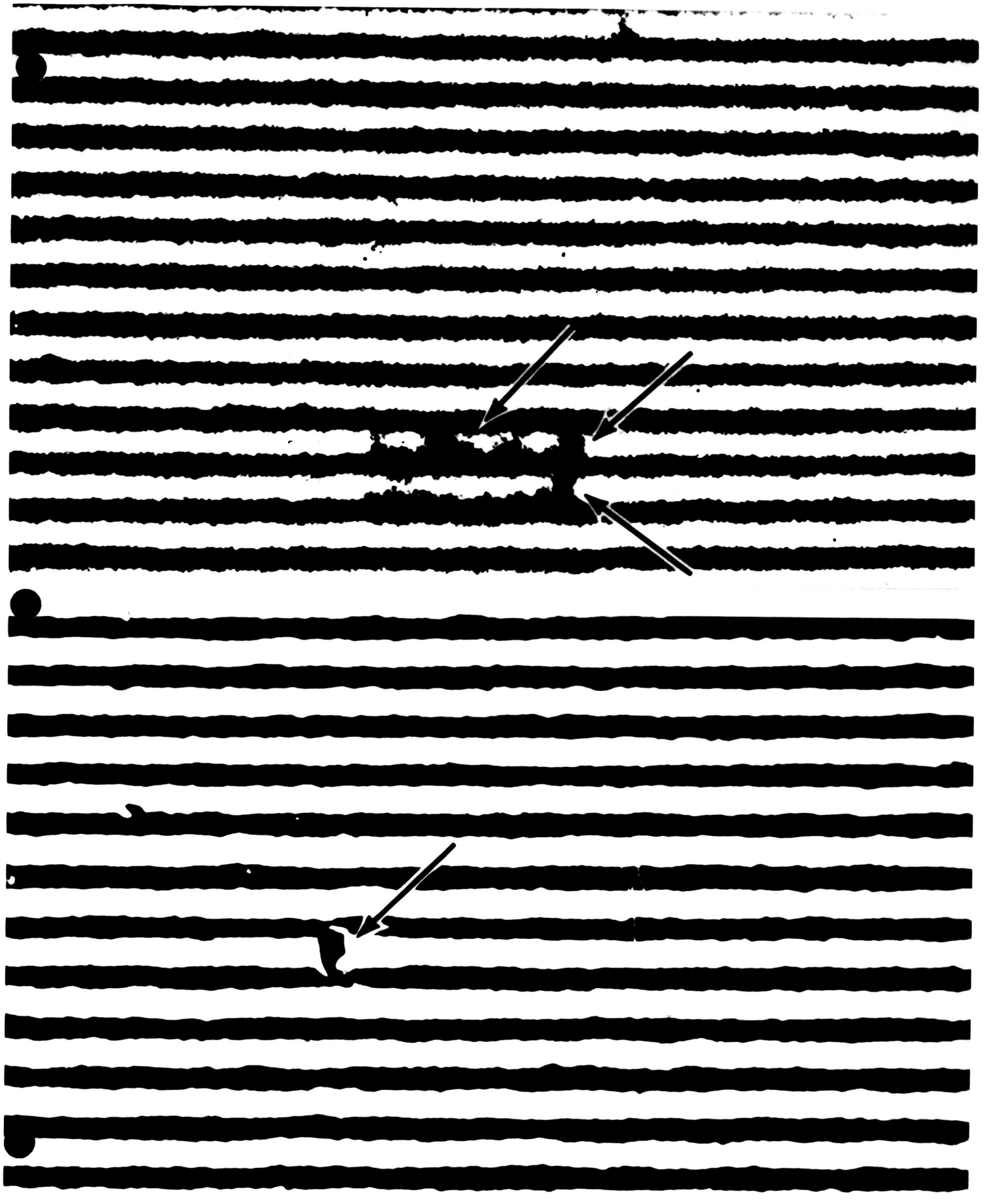
N CONTROL MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL MAGNIFICATION: X 220

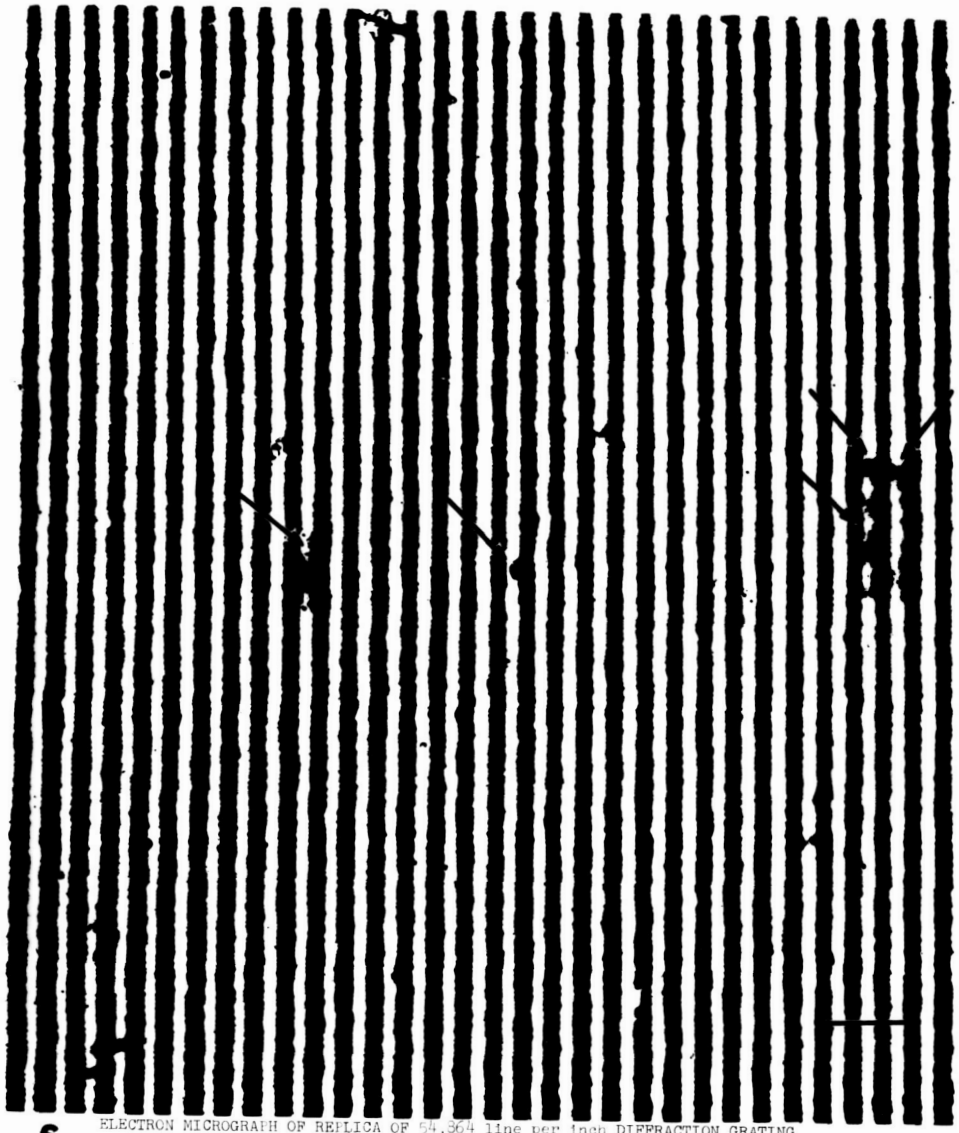


S ELECTRON MICROGRAPH OF REPLICA OF [illegible] line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X [illegible]; TOTAL MAGNIFICATION: X 50,000

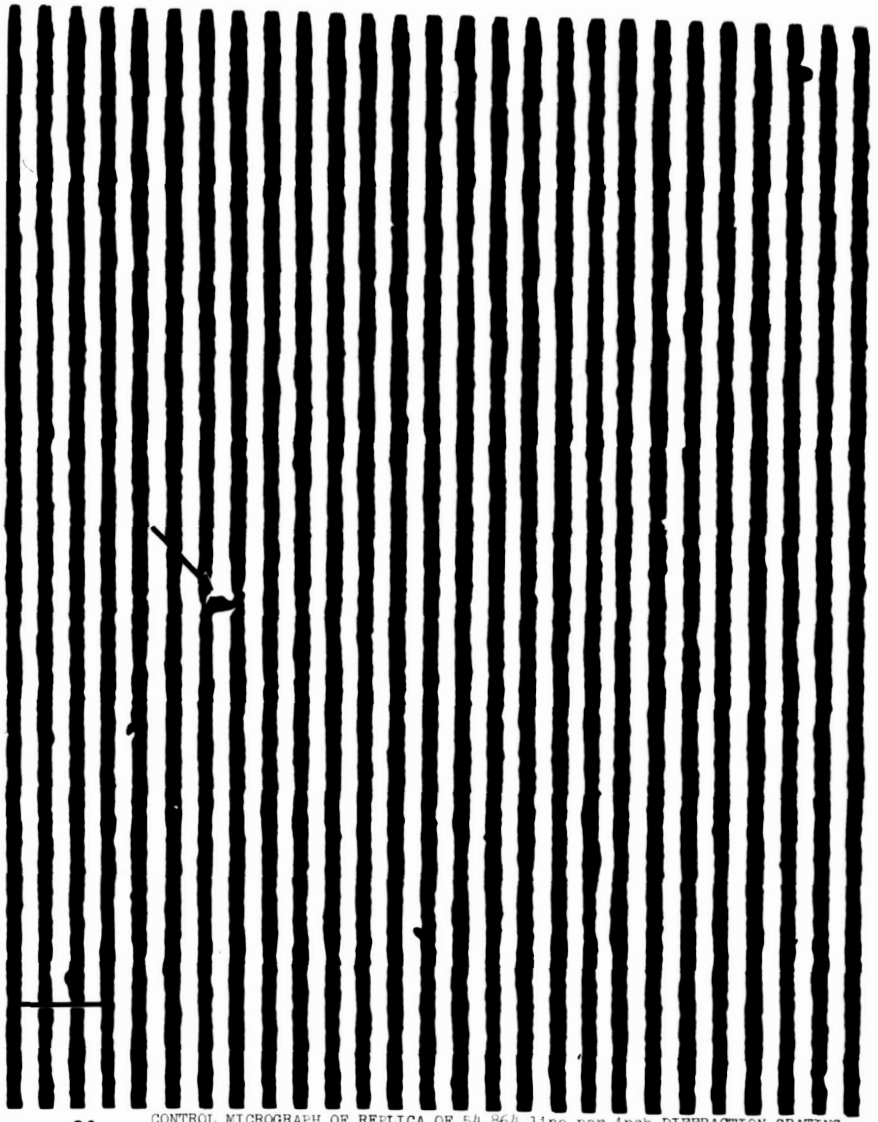


N CONTROL MICROGRAPH OF REPLICA OF 54,364 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X [illegible]; TOTAL MAGNIFICATION: X 4,400

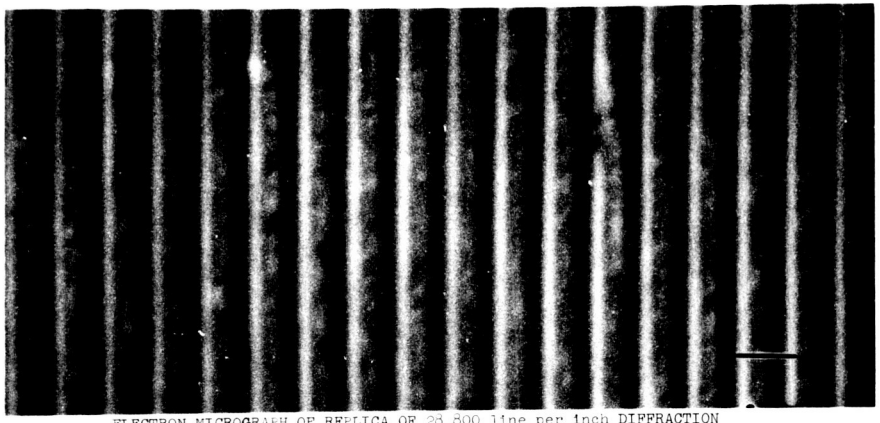




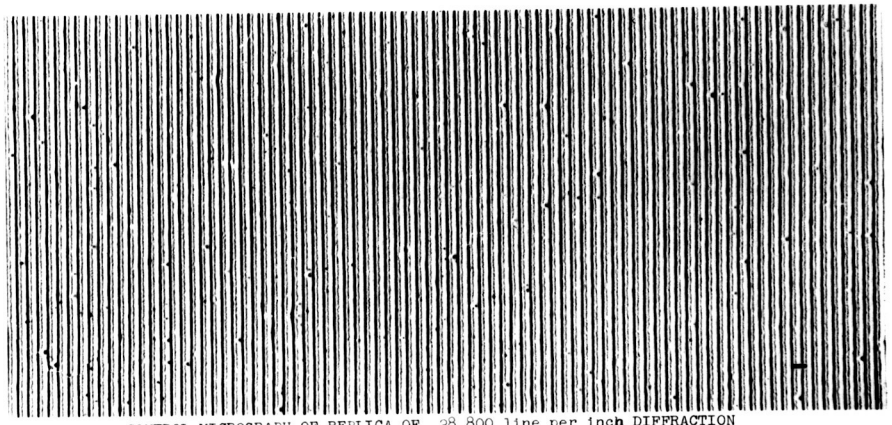
S ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE: 50 KV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 22,000



N CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELF TRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNI-FICATION: X 22,000

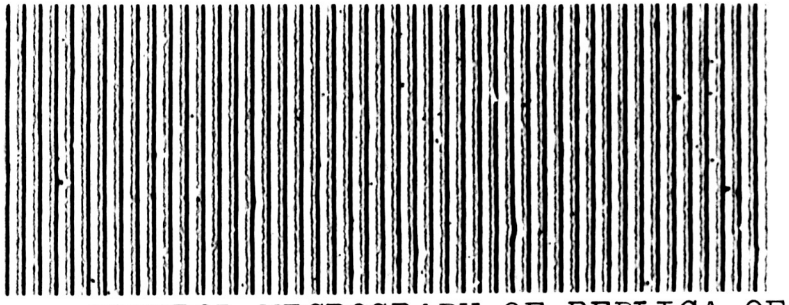


S ELECTRON MICROGRAPH OF REPLICA OF 28,800 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 2000; TOTAL MAGNIFICATION: X 17,000



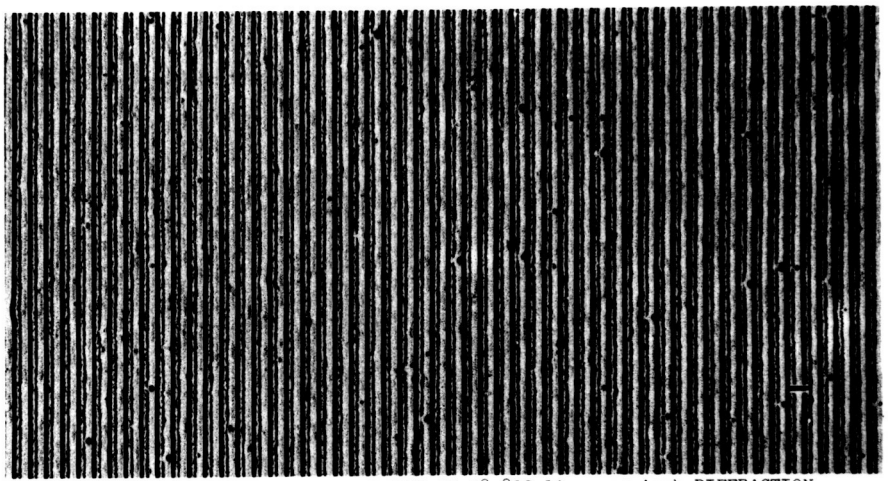
N CONTROL MICROGRAPH OF REPLICA OF 28,800 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 3,500

S ELECTRON MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; NO POLE PIECE; 50 kV ORIGINAL MAGNIFICATION:X 1200

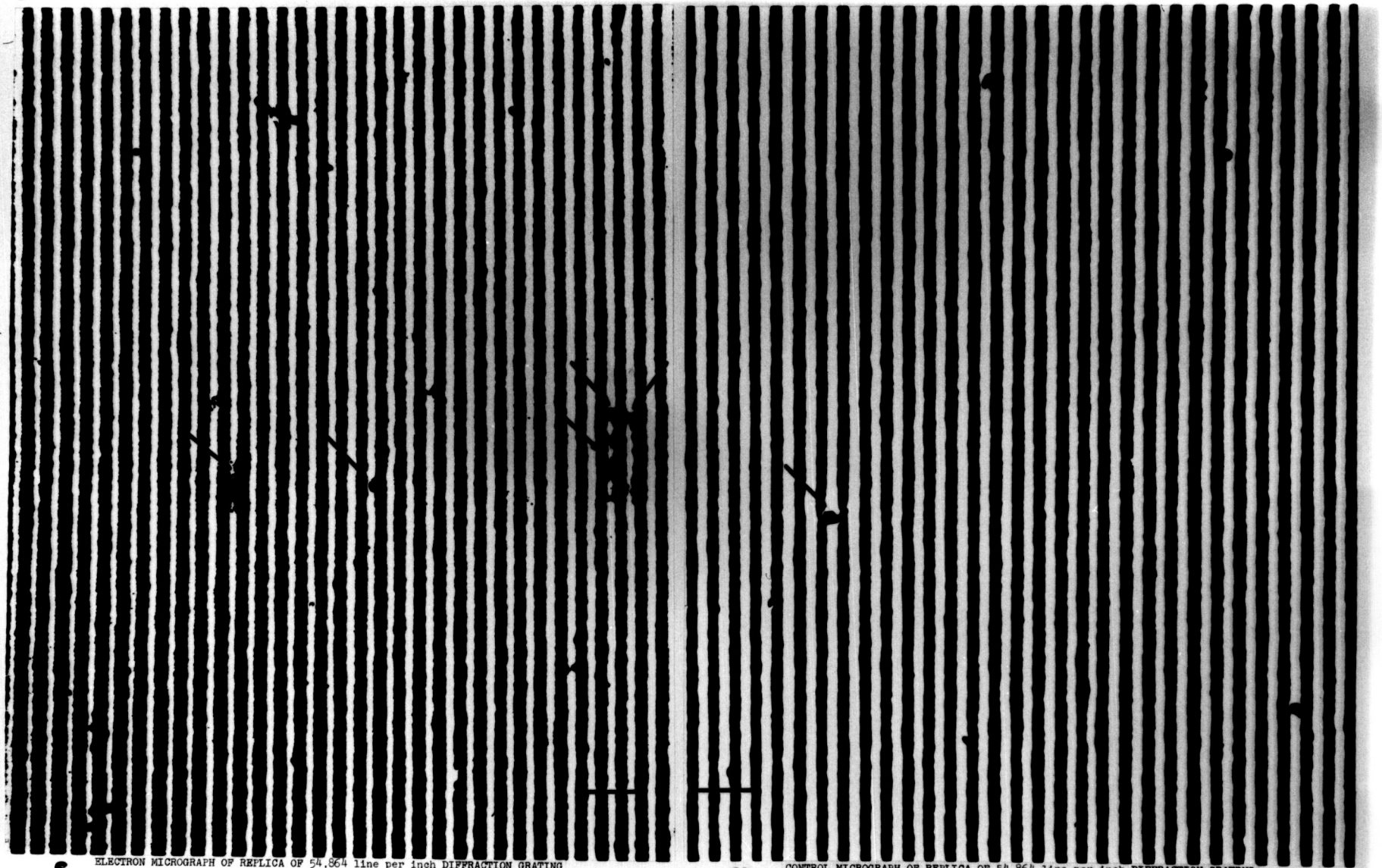


N CONTROL MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL MAGNIFICATION: X 220

S ELECTRON MICROGRAPH OF REPLICA OF 28,800 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 1000; TOTAL MAGNIFICATION: X 17,000

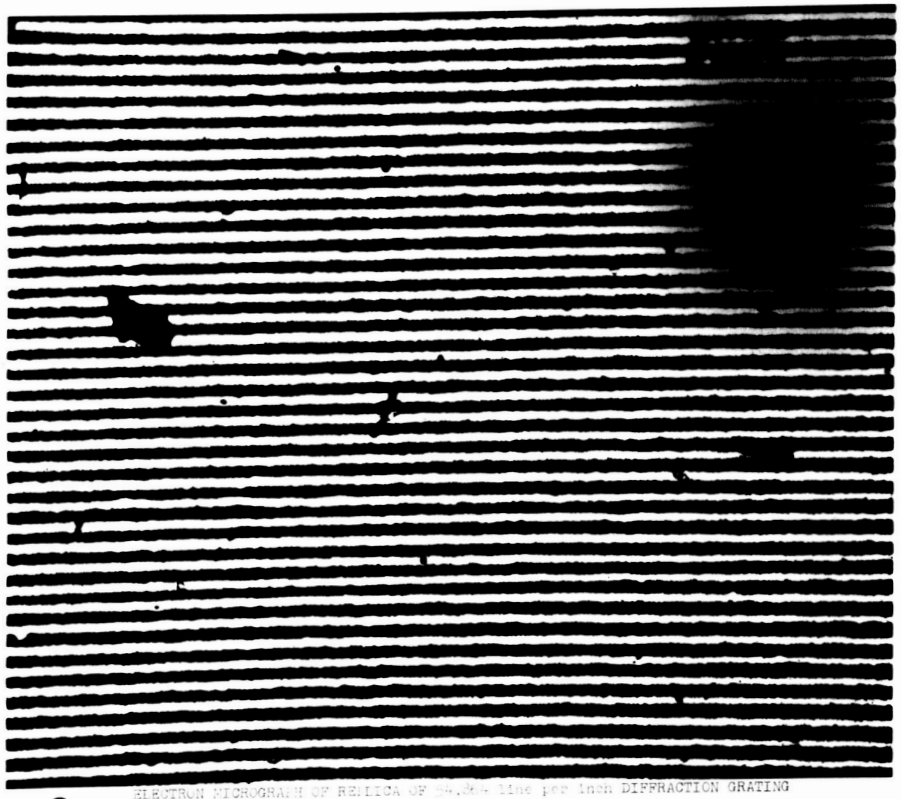


N CONTROL MICROGRAPH OF REPLICA OF 28,800 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 3,500

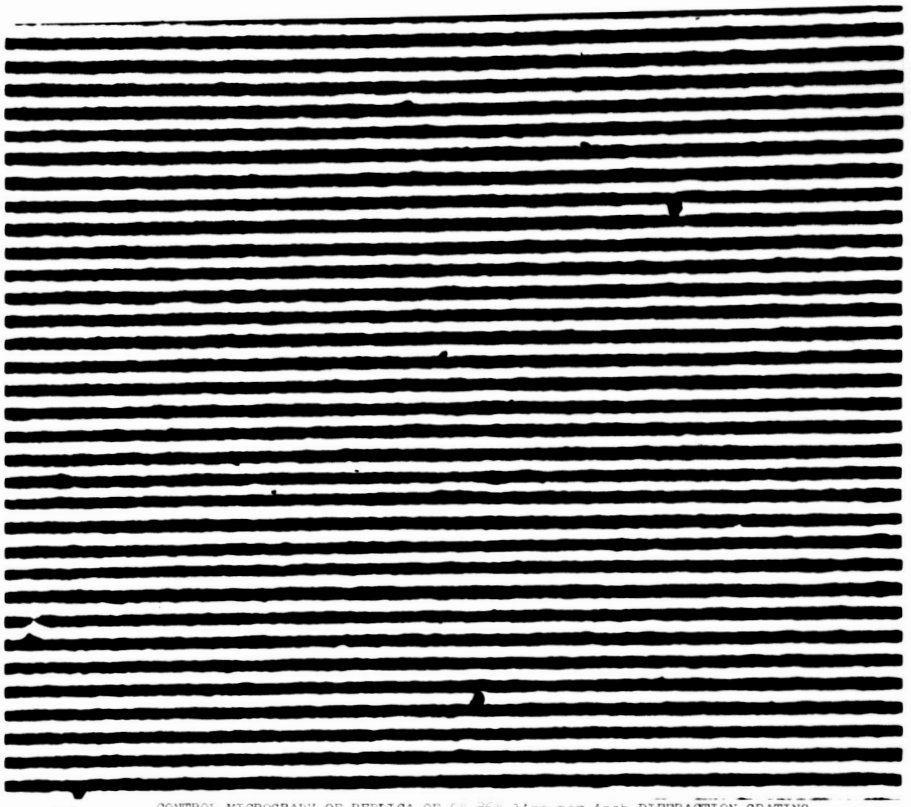


S ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE: 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 22,000

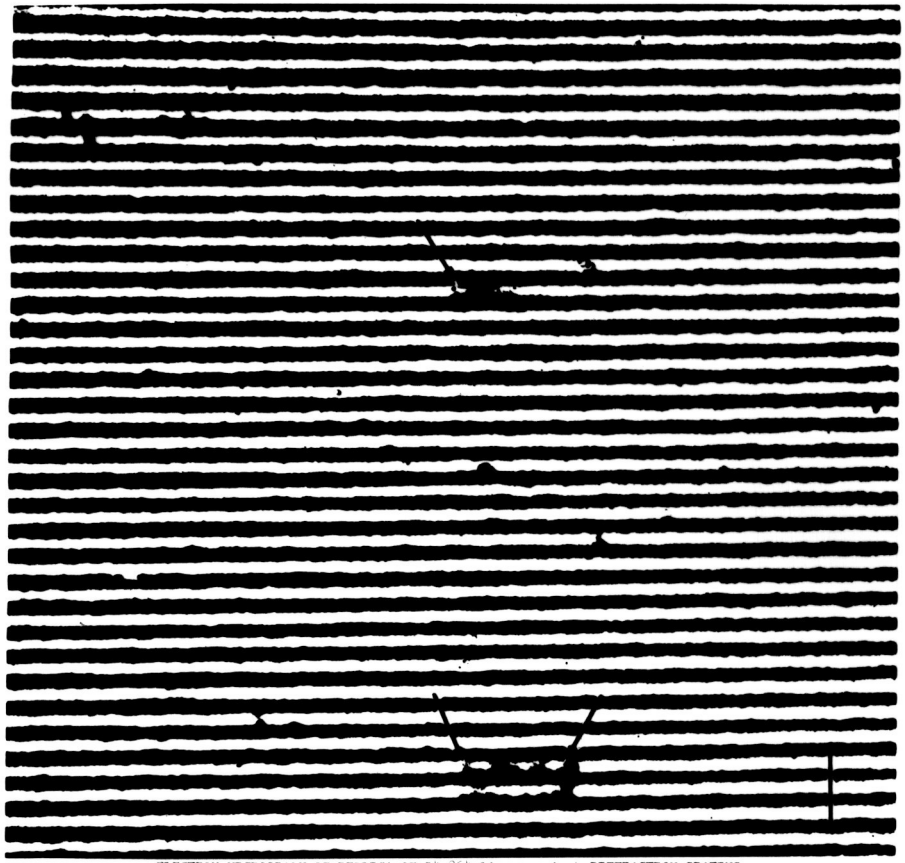
N CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 22,000



S ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH OBJECTIVE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 15,000



N CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 15,000



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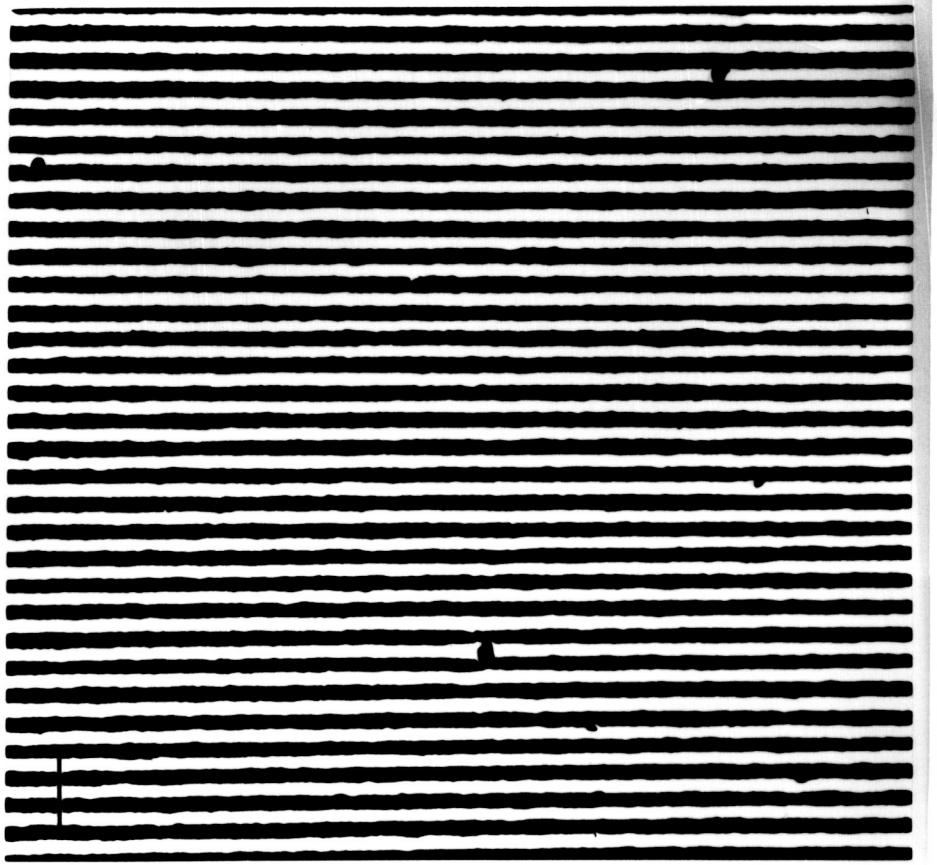
ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 50 KV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 22,000



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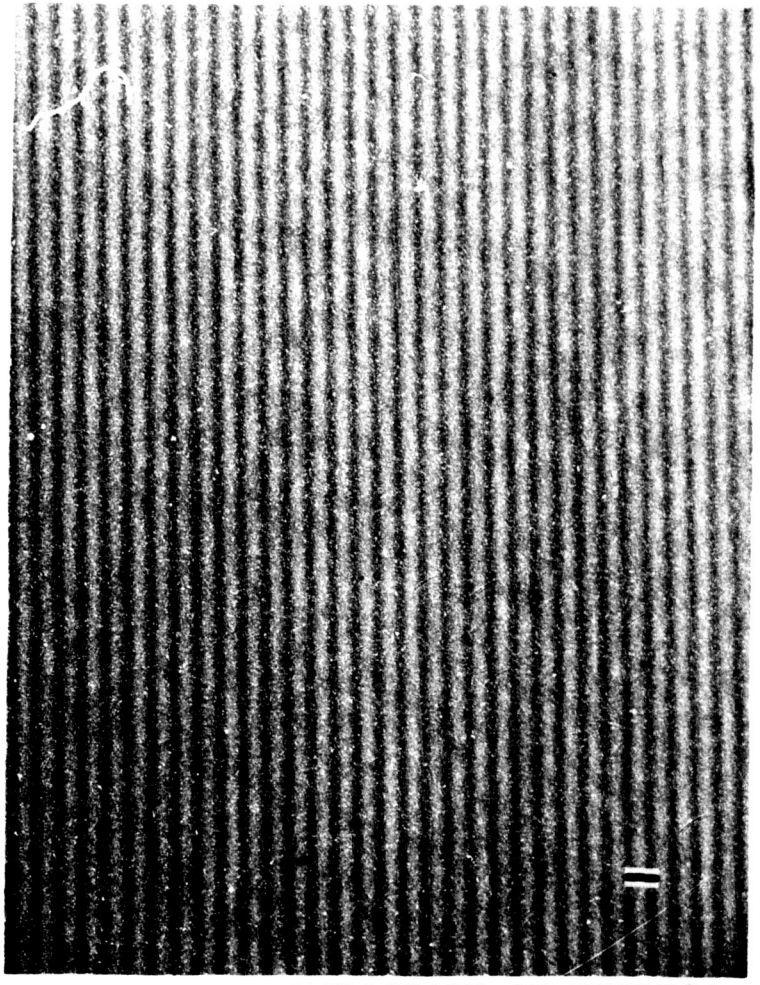
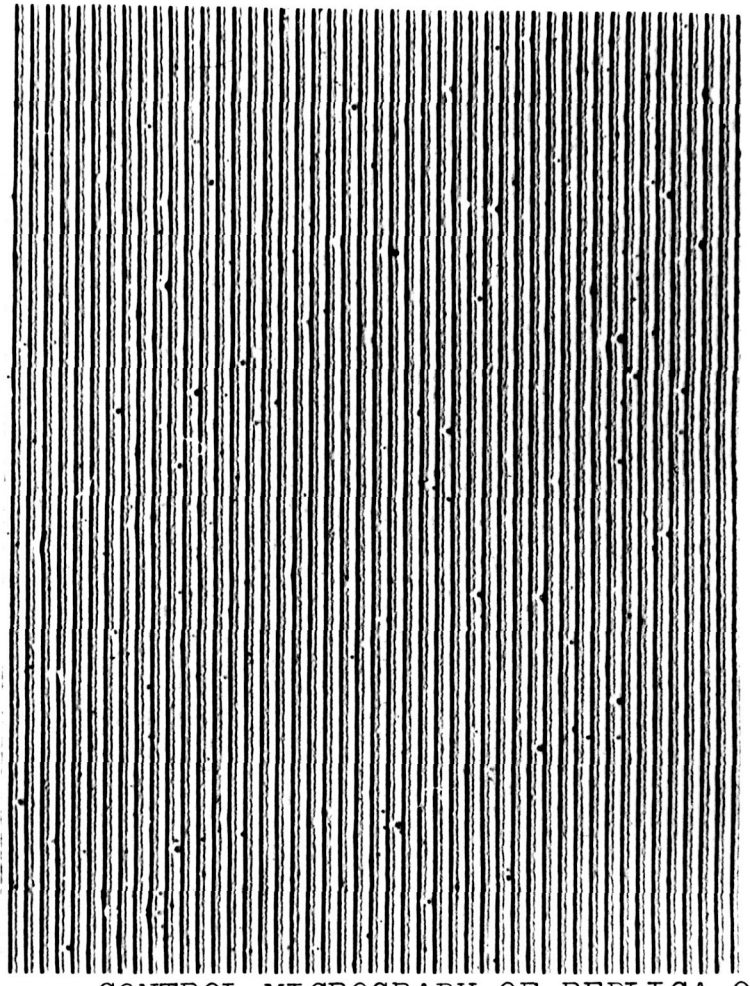
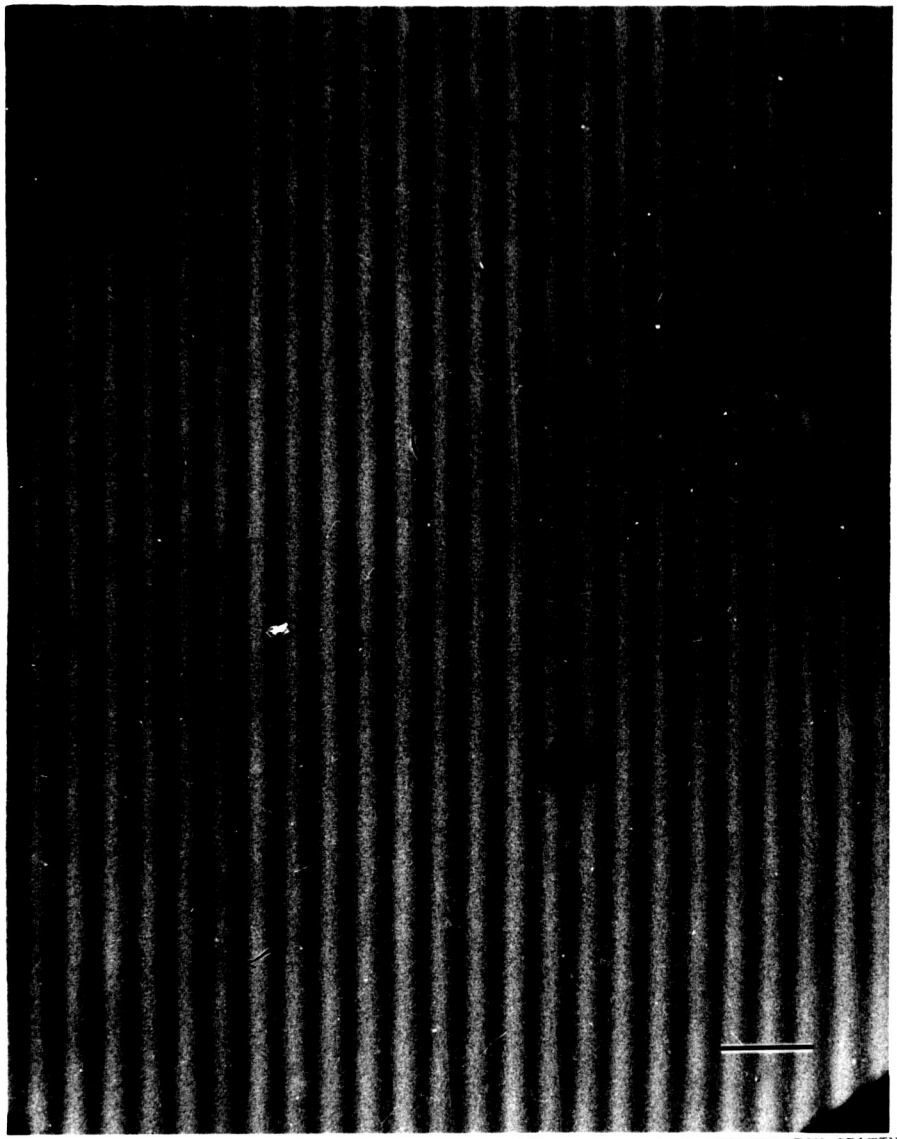
CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220; TOTAL MAGNIFICATION: X 22,000



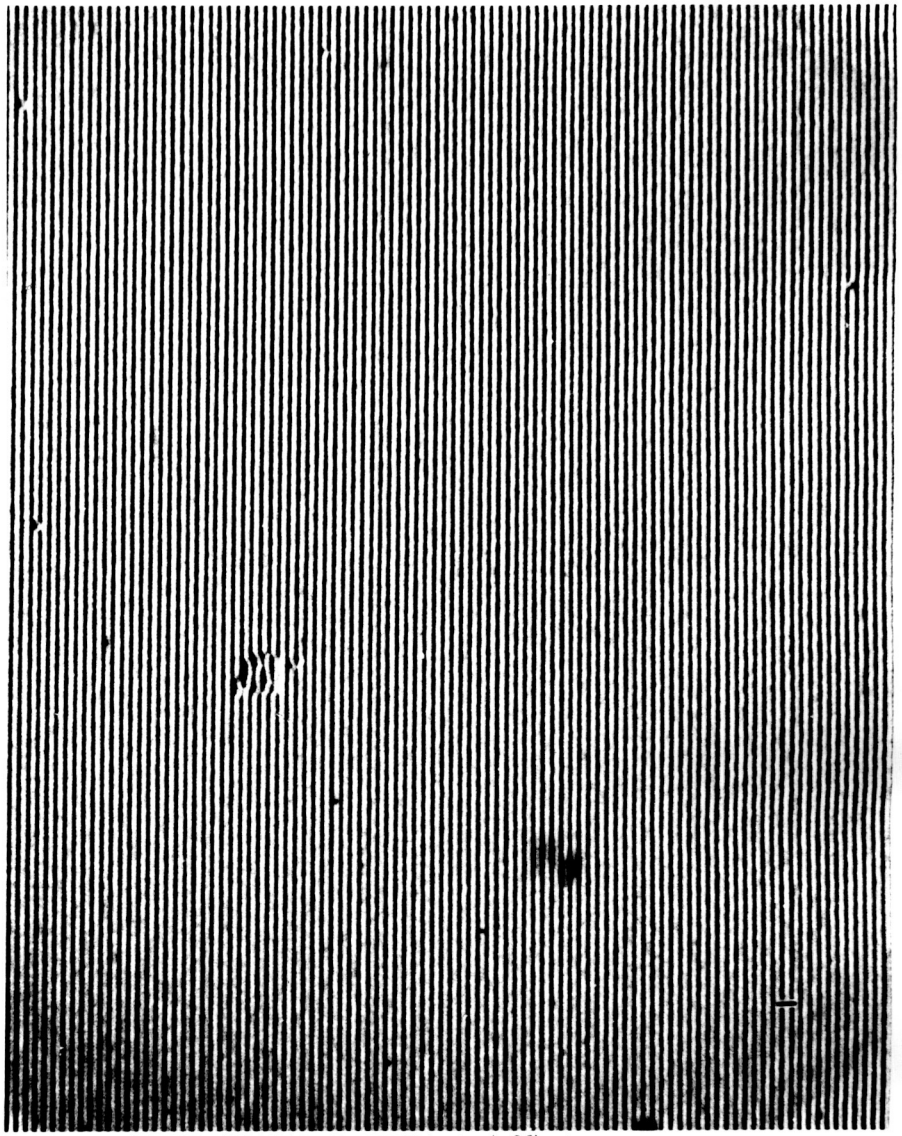
S ELECTRON MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; NO POLE PIECE; 50 kV. ORIGINAL MAGNIFICATION: X 350



N CONTROL MICROGRAPH OF REPLICA OF 28,800 line/inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL MAGNIFICATION: X 220



S ELECTRON MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH HIGH FIELD SUPERCONDUCTING LENS IN PERSISTENT CURRENT MODE; WITH POLE PIECE; 50 kV. ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 2200 TOTAL MAGNIFICATION: X 22,000.



N CONTROL MICROGRAPH OF REPLICA OF 54,864 line per inch DIFFRACTION GRATING RECORDED WITH STANDARD HIGH RESOLUTION ELECTRON MICROSCOPE WITH OBJECTIVE POLE PIECE; ORIGINAL ELECTRON OPTICAL MAGNIFICATION: X 220. TOTAL MAGNIFICATION: X 4,400.